ANGLIA RUSKIN UNIVERSITY

EMPLOYING EPIDEMIOLOGICAL APPROACHES TO ANIMAL WELFARE PROBLEMS: A TREATISE

FERNANDO JORGE RIBEIRO DA MATA

A thesis in partial fulfilment of the
requirements of Anglia Ruskin University
for the degree of Doctor of Philosophy on the basis of
published work

Submitted: August 2016

“There is no philosophy which is not founded upon knowledge of the phenomena, but to get any profit from this knowledge it is absolutely necessary to be a mathematician.”

Daniel Bernouli

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This thesis is also dedicated to the memory of my father Joaquim Fernando da Mata, my brother João Emídio Ribeiro da Mata and my brother in law Júlio Manuel Costa Lourenço.

(Fernando Jorge Ribeiro da Mata)

August 2016

ANGLIA RUSKIN UNIVERSITY

ABSTRACT

FACULTY OF SCIENCE & TECHNOLOGY

DOCTOR OF PHILOSOPHY

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By FERNANDO JORGE RIBEIRO DA MATA

AUGUST 2016

From the point of view of an animal welfare scientist, animal welfare science is seen as transdisciplinary as it establishes a conceptual framework, using disciplines shared by other scientists (*e.g.* physiology, anatomy, behaviour, nutrition and pathology). From the point of view of other scientists, animal welfare is interdisciplinary as other scientists collaborate in the field, bringing expertise in their disciplines.

This thesis aims to contribute to knowledge by demonstrating the benefit of applying a coherent framework of epidemiological standard techniques, to address a variety of animal welfare issues. The objectives are: 1 - to explore the different epidemiological study designs and biostatistical approaches and put them in an animal welfare scientific context, developing a coherent framework of useful standard techniques; 2 - to identify, discuss and assess novel animal welfare risk factors in a full range of animal settings: production, captive, companion and equine.

The potential offered by different epidemiological study designs and analytical procedures is explored and put in an animal welfare context. The illustration of this integration is made using the submitted papers and therefore the methodologies used therein are discussed and justified. The full range of epidemiological study designs is used with the application of several biostatistical approaches. This fulfils the first objective of this thesis.

The identification of different types of risk factors posed to animal welfare (genetic, environmental, husbandry and management) is one of the advantages in the use of epidemiological approaches to research animal welfare science. The identification and discussion of risk factors in a range of species (farm, companion, equine and captive) after the research contained in the publications submitted with this thesis, fulfils the second objective of this thesis. Other advantages in the use of epidemiological approaches to research animal welfare science are also discussed: introduction of standardised procedures allowing further studies; use of complex and real animal settings outside the laboratory environment; and contributions to animal welfare assessment.

Key words: Animal welfare, Biostatistics, Epidemiology, Risk factors

Contents

Glossary of Terms

ANOVA - Analysis of variance

BLUP - Best Linear Unbiased Prediction

DOI - Digital Object Identifier

GEE - Generalised Estimating Equations

GLM - General Linear Models

GLMM - Generalised Linear Mixed Models

GsLM - Generalised Linear Models

UK - United Kingdom

URL - Uniform Resource Locator

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A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy on the basis of published work

Chapter 1 Introduction

1.1. Introductory notes

This thesis for the degree of Doctor of Philosophy on the basis of published work is based on research I have led and/or made a significant contribution to, between 2010 and 2015. I have a background in animal science both as a practitioner and as an academic. After postgraduate training in both animal science and applied mathematics, I have developed academically as an animal welfare epidemiologist (full academic qualifications and professional background can be consulted in section 1.5). I have chosen nine publications to submit as part of this thesis (Appendix I), from my complete list (Appendix III) based on their quality (see section 3.2 for quality based on journal standings), thematic coherence (see section 3.4 for thematic and methodological interrelationships among the publications), and to demonstrate a significant and sustained contribution to knowledge (see section 3.5).

This introductory chapter contextualises the emergent integration of animal welfare science and epidemiology as the theme of this thesis (section 1.2). This is followed by the justification of the relevance of the topic as a valid field for investigation, and the statement of the research aims and objectives (section 1.3). The structure of the thesis is also outlined (section 1.4) and the publications part of this thesis are listed with reference to its genesis (section 1.5).

1.2. A case for the emerging integration of animal welfare science and epidemiology

In 1978 Lilienfeld regarded epidemiology as a discipline on its own developed over the previous 50 years. At that time no consensual epidemiology definition existed (Lilienfield, 1978). Nevertheless, he recognised some common themes and referred to epidemiology as a discipline “concerned with deriving biological inferences from studies of population groups dealing with a disease phenomenon”. He also recognised the contribution of established disciplines, such as biostatistics and sociology, in these inferences.

More recently, Last (2001, p. 62) defined epidemiology as “the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems". The different terms used in this definition are explained in Table 1.

Table 1 – Meaning of the different terms used in the definition of epidemiology (Last, 2001).

Study	Surveillance, observation, hypothesis testing, experiments
Distribution	Analysis by time and/or place (space)
Determinants	Physical, biological, social, behavioural factors
Health-related states/events	Diseases, threats, causes, reactions to treatments, prevention
Specified populations	Populations with identifiable characteristics

Lato sensu, animal welfare science "describes a potentially measurable quality of a living animal at a particular time" (Broom, 2011). This definition includes the capacity the animal has to cope with the environment and exposure to stress, after satisfaction of needs also referred to as the five freedoms, and the human obligations towards animals through the animal rights.

Fraser (2009) discussed the different scientific approaches and the underpinning themes in animal welfare assessment, concluding these can be clustered in three main areas: health and functioning, the affective state and the ability to live natural lives. Research within each of these three different areas has given rise to different and evolving scientific research methods, designed to assess and improve animal welfare.

The lack of integration resolving animal health and welfare problems has been identified as an issue (Houe, 2003; Rushen, 2003; Broom, 2006). The potential advantages of using epidemiology in animal welfare research are substantial (Willeberg, 1991, 1997; Rushen, 2003; Green & Nicol, 2004; Broom, 2006; Barber, 2009; Millman *et al.*, 2009; Collins *et al.*, 2010; Collins, 2012; Collins & Part, 2013; Paton, Martin & Fisher, 2013; Mendl *et al.*, 2016), and have been used with increased frequency with respect to research focusing on farm animals (Collins *et al.*, 2010). Collaboration between scientists of different fields, with different specific expertise is advantageous in the advancement of science (Hall *et al.*, 2008). It is an underpinning fact of this thesis that it is important for scientists to gain and share knowledge beyond their specific discipline of expertise and develop integrative skills. Epidemiological approaches can play a significant role in achieving this (Rushen, 2003).

Team work is an increasingly important attribute in science and research, and has been recognised by research funders as beneficial in advancing knowledge (Hall *et al*., 2008; Veissier & Miele, 2014). Team science is however an emerging discipline where the discussion of conceptual and descriptive aspects is still progressing (Choi & Park, 2006; Hall *et al*., 2008; Stokols *et al*., 2008).

There is still some confusion with terminology in describing different forms of team work relevant to the advancement of science (Weaver, 2008). Some authors do not differentiate forms of team work. Rosenfield (1992) and Choi and Pak (2006) review terminology and define multidisciplinarity as the parallel but not integrative study, by different disciplines; the addition of knowledge to disciplines provided by scientists of different expertise (*e.g.* the soil may be studied by ecologists with emphasis on its biology, while civil engineers study its plasticity, and agronomists its chemical properties, without necessarily collaborating with each other). Interdisciplinarity is the integration of disciplines, with each adding specific inputs, to establish new levels of knowledge; coordination of analysis, synthesis and harmonization of links between disciplines to provide new knowledge (*e.g.* collaborative work between chemists, botanists and agronomists to create a new agrochemical). Transdisciplinarity provides holistic views and the sharing of conceptual frameworks between disciplines (*e.g.* medicine is a transdisciplinary science that utilises chemistry, biology, and many others). The key words “addition”, “interaction” and “holistic” respectively, differentiate these concepts (Choi & Pak, 2006). The generic term “multiple discipline” may be the most appropriate when the interaction between disciplines is more complex (Choi & Pak, 2006).

Shen (2008) refers to the importance of interdisciplinarity with its capacity to empower the acquisition of knowledge. Further, Nash (2008) and Stokols *et al.* (2008) recognise the increasing importance of individuals with this integrative capacity. The need for highly specialised individuals to advance the different branches of science can create a deficit of integrative capacity, creating a concomitant requirement for multi-skilled individuals who are experts in integration and play a determinant role in team work. The understanding that integration is important in research has been surging and creates in researchers an appetite for different disciplines (Houe, 2003; Croney & Millman, 2006; Hall *et al*., 2008; Veissier & Miele, 2014).

The benefits of integration in team work has been emphasised to justify the contribution to animal welfare of social sciences to complement approaches from the natural sciences (Whay, 2007; Veisser & Miele, 2014), *e.g.* to relate the perception of animal welfare from natural scientists and the public in general (Veissier & Miele, 2014). When scientific specialization leads to the establishment of a discipline on its own, this is a sign of the growing interest in the subject, displayed by the scientific community. The natural sciences' scholars have been leading research in animal health and welfare, but social scientists may also get involved (*e.g.* animal welfare in farm animals may imply additional production costs which can be supported by a growing share of consumers prepared to pay this extra cost). This collaboration would have the advantage of allowing blockages imposed by concurrent points of view to be overcome (Lund *et al*., 2006; Whay, 2007; Wilkinson *et al*., 2011; Veissier & Miele, 2014).

Overlap between sciences is frequent, allowing scientists in the different fields to communicate and co-operate. Green and Nicol (2004) reviewed the potential advantages of co-operation between epidemiologists and welfare scientists, and made evident the need for co-operation with ethologists and animal behaviour scientists. Fraser (2010) highlighted the potential collaborative advantages between welfare scientists and conservation scientists.

Animal welfare science can be seen as transdisciplinarity as it establishes a conceptual framework shared horizontally by the animal welfare scientist. Animal welfare scientists with a diverse background work to identify and assess animal welfare issues, and research their causes to find solutions (Edwards, 2007; Leeb, 2011; Veissier & Miele, 2014, Mendl *et al*., 2016).

Through interdisciplinary work, animal welfare can progress as a science: collaboration with scientists with expertise in specific aspects of the research process, not completely dominated by animal welfare scientists. Epidemiologists have a very important role to play in animal welfare. The effect of risk factors on health and welfare are better studied in integrated research groups using holistic approaches (Houe, 2003; Rushen, 2003; Whay, 2007; Barber, 2009; Wilkinson *et al.*, 2011; Mendl, 2016).

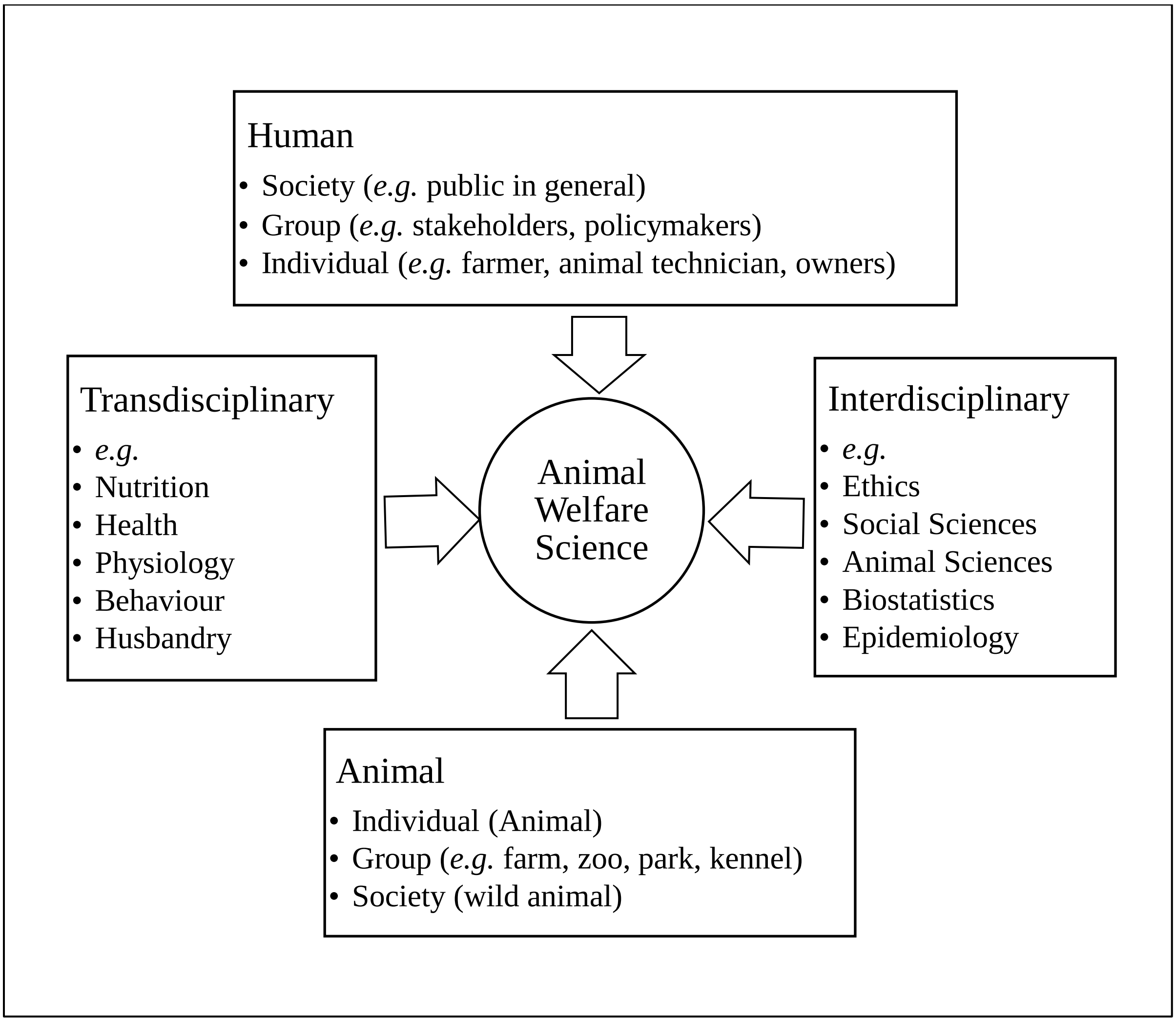
Vertical cooperation of the civil community facilitates efficiency, therefore animal welfare scientists must work directly with people on the ground (whether farmers, animal collection keepers, animal industries, or pet owners) (Edwards, 2007; Leeb, 2011; Veissier & Miele, 2014, Mendl *et al*., 2016). Animal welfare scientists need also to co-operate with a variety of stakeholders involved in the different aspects of the animal industries; and with policymakers to organise, create and disseminate rules and legislation that implement an ethically recognised practice (Edwards, 2007; Leeb, 2011; Veissier & Miele, 2014, Mendl *et al.*, 2016).

Animal welfare is a science where interdisciplinary work and the vertical integration of the animal can also be considered. Rushen (2003) and Millman *et al.* (2009) highlighted the importance of collaboration between veterinary epidemiologists and animal welfare scientists. Veterinary epidemiologists are trained to investigate the prevalence and incidence of disease in space and time, and also to identify risk factors. Animal welfare scientists tend to investigate welfare issues using a hypothetical deductive approach within a laboratory and/or controlled environment and are therefore capable of identifying human and environmental risk factors with impact on animal welfare (Rushen, 2003; Edwards, 2007). Veterinary epidemiologists are trained to perform studies in more complex settings (*e.g.* farms, parks) where interactions between risk factors exist. In complex settings, bias is limited by the ability to create study designs and use advanced analytic approaches that consider these interactions. Veterinary epidemiologists lack however, training in animal welfare measurement and evaluation, which justifies the collaboration between the two groups (Edwards, 2007; Whay, 2007; Millman *et al*., 2009). Also as Broom (2006) refers that there is an under-estimation of the impact of health on welfare by ethologist, and of animal behaviour as a welfare indicator by veterinarians.

The studying of the animal in the wild is also a key point in this vertical integration. It allows the study and identification of functionally relevant behaviours in the environment of evolutionary adaptation (Barnard & Hurst, 1996). These can be used to evaluate the adaptive trade-offs in the natural environment and in captivity which will form the basis of animal welfare assessment, avoiding anthropomorphisms (Barnard & Hurst, 1996). Figure 1 summarises the integration of the different actors that make the animal welfare science.

In 1991 Willeberg is probably one of the first scientists to relate animal welfare with veterinary epidemiology. He claims an important role to be played by veterinary epidemiology in assessing animal welfare, as the identification of risk for prevention of disease is a basic animal welfare activity (Willeberg, 1997). The investigation of incidence, prevalence and risk factors is part of the activity of quantifying animal welfare issues.

Figure 1 – The making of animal welfare science. At the horizontal level we have the transdisciplinary (diverse subject knowledge) animal welfare scientist. The animal welfare scientist collaborates interdisciplinary (with scientists of different expertise). At the vertical level we have several levels of human collaboration (branching out) including individuals, groups of individuals and the society in general. Animal welfare science can also consider the vertical integration of the animal: by comparison of the individual with peers in the considered setting and by studying the animal in natural settings for differentiation of abnormal behaviour mitigating anthropomorphisms.



During the 1980s it became clear that animal welfare could not be fully evaluated without a holistic approach to the animals' health status (Broom, 1986; Broom, 1988). Disease is itself a measure of poor welfare but can be a confounder if not considered when evaluating other welfare issues, such as husbandry (Rushen, 2003; Broom, 2006). Disease can be the cause of poor welfare itself, but other causes may be of higher importance with development of disease as a lateral consequence; in this case the disease can be considered as the symptom of the causal factor of poor welfare. Willeberg (1991, 1993) raises this question and introduces epidemiology as an important tool in the identification of poor welfare risk factors.

Green and Nicol (2004) discussed advantages of the use of epidemiology in animal welfare science, and indicated epidemiology as the perfect tool for the identification of environmental, management and genetic risk factors with impact on animal welfare.

To date epidemiology has been used while studying the impacts of environment and infrastructure in a variety of animal welfare settings (Paton, Martin & Fisher, 2013) including aquaculture (*e.g.* Turnbull *et al.*, 2011), companion animals (*e.g.* Collins *et al.*, 2010), animal production (*e.g.* Dewey *et al.*, 2009), animal collections and zoos (*e.g.* Carlstead *et al*., 2013) and equine (**Williams, Parrot & Da Mata, 2012**).

1.3. Exploring and evaluating the potential of epidemiological approaches to animal welfare. Research aim and objectives

The collaboration between epidemiologists and animal welfare scientists has enormous potential to advance animal welfare science. It is in the context of this developing collaborative effort between scientific disciplines that the work presented in this thesis arose. The recognised need for integration in team work, and particularly integration of animal welfare science and epidemiology, corroborates the relevance of the aim of this thesis. There is a generic framework gap that limits the adoption of epidemiological procedures to investigate causes of poor animal welfare.

The portfolio of publications part of this thesis and accumulated by the author arrive from an active research interest in the interface between epidemiology and animal welfare. While contributing to the aim of this thesis, the research performed individually and reported in each of the research outputs produced contributions to knowledge. This is done mainly through the identification of risk factors capable of impacting animal welfare, but also potentially contributing to animal welfare assessment (discussed in detail in the section 3.5).

This thesis aims to further develop the emerging integration of animal welfare and epidemiological sciences. The derived objectives are:

1. To explore the different epidemiological study designs and biostatistical approaches and put them in an animal welfare scientific context, developing a coherent framework of useful standard techniques.
2. To identify, discuss and assess novel animal welfare risk factors in a full range of animal settings: production, captive, companion and equine.

1.4. Thesis structure

This thesis is developed over three chapters, integrating the nine publications submitted with this thesis. These chapters are complemented by four appendices.

This first chapter introduced and contextualised the topic, justifying its choice and relevance, and defining aims and objectives. Chapter 2 is an essay, intended to be published on its own as a review. It establishes a framework for collaboration between animal welfare science and epidemiology. In this chapter my papers are introduced as examples of the application of epidemiological techniques in animal welfare research and the methodologies are described. Chapter 3 explores the interrelationship of the publications that constitute the portfolio for this thesis and appraises the originality and contribution to knowledge of each of them. It also highlights the advantageous use of epidemiological approaches, and the methodologies used in the papers part of this thesis are appraised. My contribution in each of the publications is acknowledged and a comment on the standing of the journals where the works were published, together with the identification of the known citations, is also made.

Appendix I comprises the nine publications part of this thesis and listed in the following section; Appendix II presents statements and declarations to fulfil the requirements of the Anglia Ruskin University Research Degree Regulations 2015, namely regarding the items c), d), e) and f) of point 4.1 of its part B; and Appendix III contains the chronology of all my publications.

1.5. Genesis and chronology of the publications used as part of this thesis.

To comment on the genesis of the publications included in this thesis it is necessary to introduce my academic qualifications and professional background. I did my first degree (animal sciences) at the normal age following secondary school and started my professional career as a dairy farm manager in a family farm. While working, I also engaged in a longlife learning process to complement the knowledge acquired, I started a second undergraduate degree in agronomy with an animal science minor studying part time.

By the end of my second undergraduate degree I had the opportunity to start a professional academic career as a lecturer and did some publications not included in this thesis. From this point I felt that I needed to complement even further my studies and decided to study applied maths (experimental statistics) in order to be prepared for a research degree.

The opportunity to do a Doctorate didn't appear once, as a family man, it was impossible to live without a salary, even in the case of being eventually given a grant. As a result the desire of doing a Doctorate was delayed.

I moved to the UK in 2006 to lecture in animal sciences at Hartpury College and had the opportunity to qualify as a lecturer (taking a post graduate certificate in teaching and learning) and further enhance my studies with a Masters in zootechnics taken by distance learning. While at Hartpury and after settling in the UK, I engaged in scholarly activity with publications arising from individual initiatives and collaborations.

As a result of my background in animal sciences and applied mathematics and my interest in animal welfare, I have been researching in the interface of these sciences: the application of advanced biostatistical techniques to evaluate risk factors capable of impacting on animal welfare, and therefore the application of epidemiological techniques in animal welfare research.

This doctorate by published work was found to be the way forward in my academic career once I have the necessary skills to research and be published.

Table 2 summarises my academic achievements to date and Table 3 my professional background.

The list of publications submitted as part of this thesis is also introduced. This includes their DOI or when not available their URL.

Table 2 – Academic qualifications by the author of this thesis

Name of Institution	Qualification and subject	Date
Technical University of Lisbon	MSc. Zootechnics	2013
University of the West of England	PGCert. Teaching & Leaning in HE	2009
Technical University of Lisbon	PGDip. Experimental Statistics	2005
Technical University of Lisbon	Lic. Agronomy with Animal Science	2002
Polytechnic Inst. of Castelo Branco	Bac. Animal Science	1989

Note: MSc – Master of Science PGCert – Post-Graduate Certificate; PGDip – Post-Graduate Diploma; Lic. – Licenciatura; Bac. Bacharelato

Table 3 – Professional career by the author of this thesis

From	To	Employer	Position Held
01/05/2016		Wrexham Glyndŵr University	Senior Lecturer
01/08/2015	30/04/2016	Hadlow College	HE Lecturer
01/09/2013	31/07/2015	Newcastle University	Teaching Fellow
18/01/2006	31/08/2013	Hartpury College	HE Lecturer
01/09/2002	15/01/2006	Polythecnic Institute of Portalegre	Adjunct Professor
01/04/1990	31/08/2002	SAP Mata & Irmão, Lda	Dairy Farm Manager

Note: SAP – Sociedade Agro-Pecuária; HE – Higher Education

List of publications part of this thesis:

Mata, F. (2015) The choice of diet affects the oral health of the domestic cat. *Animals*. 5 (1), 101-109. DOI: 10.3390/ani5010101

Mata, F., Johnson, C., Bishop, C. (2015) A cross-sectional epidemiological study of prevalence and severity of bit-induced oral trauma in polo and race horses. *Journal of Applied Animal Welfare Science*. 18 (3), pp.259-268. DOI: 10.1080/10888705.2015.1004407

Mata, F. (2014a) Evaluation of horse fitness for exercise: the use of a logit-log function to model horse post-exercise heart rate recovery. *Journal of Equine Veterinary Science*. 34 (9), pp.1055-1058. DOI: 10.1016/j.jevs.2014.06.004

Mata, F. (2014b) Analysis of predisposition factors for limb amputation in dogs with survival analysis in those diagnosed with appendicular cancer. *The Veterinary Nurse*. 5 (7), pp.406-411. URL: http://www.theveterinarynurse.com/cgi-bin/go.pl/library/ article .cgi?uid= 106180; article=_5_7_406_411

Mata, F. (2013) Mastitis vaccination in dairy cattle: a meta-analysis of field case-control trials. *Revista Portuguesa de Ciências Veterinárias*. 108 (585-586), pp.17-22. URL: http://www.fmv.utl.pt/spcv/PDF/pdf12_2013/17-22.pdf

Mata, F., Lam, A. (2013) Investigating the relationship between feed and helminthic burden of captive birds of prey in Hong Kong. *Zoo Biology*. 32 (6), pp.652-654. DOI: 10.1002/zoo.21103

Mata, F., Mwakifuna B., (2012) Comparative mortality and predation in relation to egg production traits of Rhode Island Red, Black Australorp and Hyblack laying hens in scavenging production systems of rural Malawi. *British Poultry Science*. 53 (5), pp.570-575. DOI: 10.1080/00071668.2012.736612

Mata, F., Williams J., Marks F. (2012) Investigation of factors associated with the probability of racehorses being pulled up in steeplechase races at Cheltenham racetrack. *Comparative Exercise Physiology*. 8 (2), pp.95-101. DOI: 10.3920/CEP12012

Williams J., Parrot R., Da Mata, F., (2012). Effect of manual and motorised dental rasping instruments on Thoroughbred’s heart rate and behavior. *Journal of Veterinary Behavior: Clinical applications and research*. 7 (3), pp.149-156. DOI: 10.1016/j.jveb.2011.08.007

Chapter 2 A Framework for Using Epidemiology in Animal Welfare Science

2.1. Introduction

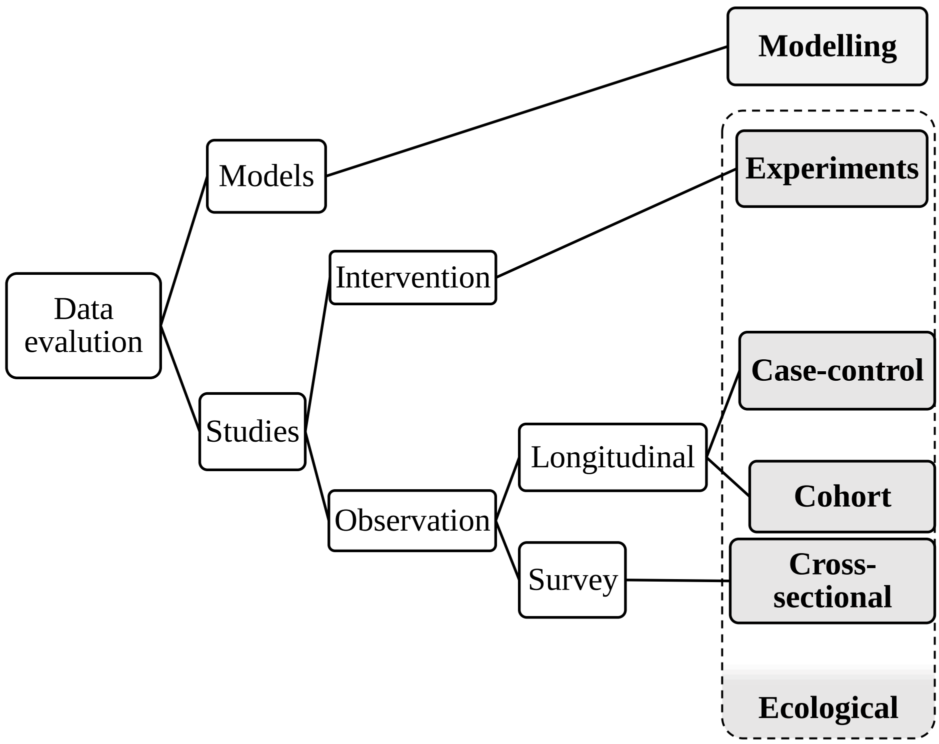
As outlined in chapter 1, there is enormous potential for the effective collaboration between epidemiologists and animal welfare scientists. In this chapter a framework of relevant techniques and methodologies is presented. This is aimed to encourage animal welfare scientists to adopt these approaches when advantageous to further allow the integration of animal welfare and epidemiological sciences. In the different sections of the chapter the different epidemiological study designs and analytical procedures are explored and put in an animal welfare scientific context. The biostatistical approaches used in the different study designs are also put in perspective. The illustration of this integrative framework is made using works in the public domain. Finally the papers part of this thesis are also introduced and therefore the methodologies used therein are put in an epidemiological context.

2.2. Applying epidemiology in animal welfare science

The concepts of debate among scientists at least since Hippocrates but the quantitative analysis started to introduced in the 18th century only, by scientists such as Arbuthnot (1665-1735), Bernoulli (1700-1782) and d'Alembert (1717-1783) (Matthews, 2016). The concepts of control group, randomization and placebo to overcome bias were discussed from the 18th century, however it was only in the end of the 19th century, with the creation of the Biometric School at The University College London, that statistical techniques such as curve fitting, goodness of fit tests and correlation measurement were developed by Pearson (1857-1936) (Matthews, 2016). The father of modern clinical trials is the British statistician Austin Hill (1897-1991) and today's researchers have just refined Hill's ideas on trial design (Collier, 2009; Matthews, 2016). Fisher (1890-1962) was contemporary to Pearson but younger; he develop the work of Pearson sometimes with divergence of thoughts. Fisher's book the Design of Experiments (Fisher, 1935) linked experimental design and estimation and was followed in genetics, agronomy, economy, psychology, medicine and many other sciences (Aldrich, 2007). From the second half of the 20th century, after the development of methodology for the identification of health risk factors by observation of subjects in real life situations, rather than in an experiment, epidemiology evolved. The development of cohort, cross-sectional and case-control studies resulted from this evolution and gave rise to epidemiology as a science on its own (Pearce, 2005).

Modern epidemiology uses a variety of research designs to plan the collection of data. There is however no definitive classification, which may vary with the purpose (Pearce, 2012). Some variability may be observed, function of different ‘classification axes’ (e.g. incidence or prevalence studies, requiring or not sampling, having a dichotomic, categorical or continuous outcome measure) (Pearce, 2012). The majority of the studies have in fact mixed features (Silva, 1999). The classification adopted herein (Figure 2) is adapted from Thrusfield (2005) and chosen as suitable in animal welfare epidemiology, as per exemplification done in the following sections.

Figure 2 – A possible classification of the different types of designs (shadowed boxes) to use in animal welfare epidemiology, with indication of differentiating characteristics (clear boxes). Ecological studies are a particular type of observation studies with a collective unit of analysis rather than individual. Adapted from Thrusfield (2005).



In animal welfare epidemiology data can be evaluated distinguishing between ‘models’ and ‘studies’. Models are created to allow the prediction of temporal and spatial behaviour of disease; studies aim to identify characteristics of the environment or the population, differentiating progression of the disease. The different studies are classified differentiating between ‘intervention’ (researcher having an active role in manipulating the individuals and the environment in a laboratory) and ‘observation’ (researcher with a passive role, studying the environment and the individuals in real settings without any manipulation but defining procedures for collection of data).

Observation studies can be broken down in two types accordingly the temporal collection of data. If made in the present in a defined time span we have a survey and the observation studies are classified as ‘cross-sectional’; if the data is collected over time the observation studies are classified as ‘longitudinal’. Observation longitudinal studies can be further broken down into ‘cohort’ and ‘case-control’ studies. Cohort studies are done prospectively, *i.e.* from the point of view of the researcher the conditions are set in the present and data is collected in the future; Case-control studies are done retrospectively, *i.e.* from the point of view of the researcher data is collected from the past.

If the data is collected from groups of individuals, *i.e.* a collective unit of analysis (*e.g.* farm, park, herd), the study is named ‘ecological’. An ecological study can be done in the present, retrospectively or prospectively and therefore can be seen, respectively as an ecological cross-sectional study, an ecological cohort study or an ecological case-control study.

These consideration are further developed in each of the following sections where each of the particular types of study will be discussed with more detail.

2.3. Modelling

Mathematical modelling has been applied in biological sciences, and can be defined as the mathematical representation of quantitative events, to allow their predictions (Oliveira & Hilker, 2010). Cohen (2004) explored synergisms between biological and mathematical sciences and, while anticipating the future of the relationship, identified monitoring of deviations in living systems (epidemics and physiological or ecological pathologies) as an area of potential major development. In epidemiology, the technique is used mainly to model patterns of disease occurrence, to allow the prediction of spatial and temporal spread, benefiting surveillance and the adoption of control strategies (Thrusfield, 1995).

There are still not many mathematical models applied to animal welfare science (Lawrence, 2008; Collins & Part, 2013), however the potential is substantial (Collins & Part, 2013). Østergaard, Sørensen and Kristensen (2000); Aerts, Wathes and Berckmans (2003); Thornton (2010); and Bruijnis *et al*. (2012) are good examples of applications. Østergaard, Sørensen and Kristensen (2000) used a mathematic model to simulate the relationship between feed, health and production in dairy cows. Aerts, Wathes and Berckmans (2003) used feed, light and temperature to model growth and metabolic heat production in broilers. Thornton (2010), while discussing future perspectives of livestock production, identifies the inclusion of animal welfare parameters in BLUP (Best Linear Unbiased Prediction) animal models for estimation of breeding values.

Mata (2014a) is a mathematical modelling study where the use of the logit-log equation was proposed to model post-exercise heart rate recovery data in horses. The study introduces the logit-log equation as a parsimonious model, and the use of the half recovery time as a measure of horse fitness. The model was fitted to a set of data collected from polo ponies and is shown to have a high degree of adjustment. It is argued that this model shows potential to be used in simple portable equipment allowing quick evaluation of horse fitness.

2.4. Experimental studies

An experiment or a trial is a study made in controlled conditions. The investigator intentionally manipulates one or more ‘factors’ of interest to evaluate their effects. These are measured between groups of individuals receiving or not receiving a treatment related to a factor of interest. The ‘control’ group is composed by individuals not receiving the treatment. This group may receive a ‘placebo’ or sham treatment to avoid bias, or can also receive a different treatment and the treatments work as controls of each other. The individuals in the different treatment groups should be similar except in the treatment they are subject to. The different treatments in the factor are named ‘levels’ of the factor.

The allocation of treatments to individuals is done ideally 'blind' to the eyes of the researcher, to avoid bias; this is not always possible as some factors do not allow that flexibility. For example, when studying the effect of a bedding material in poultry the researcher can chose the different groups to allocate to the different beddings, but if the factor of interest is breed, the individuals cannot be allocated to a breed they do not belong to. These aspects are used to classify the experiments as 'randomized', 'semi-randomized' and 'non-randomized'.

In randomized experiments the individuals are allocated completely at random to the different treatment or treatments. In semi-randomized experiments there is more than one factor being analysed; if one of the factors is not allocable, different groups with equal number of individuals for each of the levels of that factor are created, and the second factor is randomly allocated within each of the groups. In non-randomized experiments the factor is not allocable. Using the previous example: in a randomized experiment one particular breed is used and randomly divided in three equal groups that are allocated to each of the bedding materials; in a semi-randomized experiment each of the three breeds is divided in three equal groups (nine groups of individuals) and the birds are allocated randomly within each of the beddings (the three breeds are represented in the three beddings); in a non-randomized experiment the three breeds are used in a particular bedding to test eventual differences between breeds.

The test of different effects can be done 'within' the same subject or 'between' subjects. Using again the same example, we may want to test the effect of the bedding material over time; different bedding materials have different properties (*e.g.* capacity to absorb humidity) and may convey different welfare measurements over time. If we include the factor time in our design with the levels 20, 40 and 60 days, we take 'repeated' or 'paired' measurements in the same individuals over time. In this example time is a 'within subjects' measure and bedding a 'between subjects' measure. The factors tested in experiments are known as fixed factors when the measurements for the different levels of that factor are taken between individuals (independent measures), and as random factors when taken within individuals (paired or repeated measures).

Experimental designs include factors of interest, but sometimes nuisance factors need to be considered in the design. In a 'full factorial' design no nuisance factors are considered and all the combinations between the levels of the different factors being studied are present. In ‘blocked’ designs one factor of interest is considered together with one or more nuisance factors: the ‘split plot’, the ‘Latin square’, and the ‘Graeco-Latin-square’ designs include respectively one, two or three nuisance factors. Blocked designs contain all the combinations between levels of the factors, but do not contain replications for the nuisance factors; the number of levels of each of the factors is equal and the treatments are allocated at random.

Blocked designs reduce the number of individuals in the experiment. They have, however the disadvantage of not allowing the study of interactions between factors. Using again the same example, if we are using three farms in the experiment and in each farm three hatching times, and if we use three different buildings with different ventilation systems in each farm, we may need to enter farm, hatching time and ventilation system as nuisance factors (Illustrated by Figure 3). A full range of other designs is found in the literature, based on the ‘combinatorics’ theory of ‘orthogonal arrays’ (*e.g.* Colbourn & Dinitz, 2006).

Figure 3 – Latin and Graeco-Latin experimental designs. Latin letters stand for three different bedding material and Greek letter for ventilation system. In the Latin square deign (left) three different farms and three different hatching dates are eventual nuisance variables. In the Graeco-Latin square design (right) ventilation system is a third eventual nuisance variable.

Hatching dates	Farm 1	Farm 2	Farm 3
1	A	B	C
2	B	C	A
3	C	A	B

	Farm 1	Farm 2	Farm 3
1	A α	B γ	C β
2	B β	C α	A γ
3	C γ	A β	B α

Experiments are statistically analysed with ANOVA like models, which are included in the family of the ‘general linear models’ (GLM). Parametric tests are used when the prerequisites are met: one, two or multiway ANOVAs, depending on the number of factors in the design; if the independence of the observations is not met, then a repeated measures

ANOVA should be used. There are alternative non-parametric approaches, namely the Kruskal-Wallis test for independent measures and the Friedman test for repeated measures. Vittinghoff *et al.* (2012) is an example of literature where the topic is comprehensively introduced and explored.

Experiments should ideally be 'complete' and 'balanced', containing all the treatments and their combinations, and with the same number of observations per treatment. Occasionally animals are withdrawn, because they need to be moved, die or other reason. If the number of observations is large, losing one animal does not have major implications and commonly the software treats this aspect by replacing a missing value with a mean value; however if the number of observations is small or the number of withdrawals high, a type II ANOVA is preferred to the traditional type III (*e.g.* Langsrud, 2003).

So far, we have looked to statistical models dealing with dependent or response variables that measure something ('continuous', 'interval' or 'scale' data). Frequently the dependent variable is not continuous, *e.g.* count of the number of times a certain behaviour was performed; and existence or not of a certain behaviour. In the first example we have a 'nominal' variable (count), in the second example we have a 'dichotomic' variable (existence or not). These type of dependent variables are analysed with a group of models known as 'generalised linear models'[1] (GsLM). These models use a 'link function' to allow the response variable to vary linearly. With counts we use link functions from the 'Poisson' family (*e.g.* Poisson, 'negative binomial', 'log'), and with dichotomic variables we use link functions from the 'binomial' family (*e.g.* 'logit', 'probit', 'complementary log-log', 'negative log-log'). These link functions are the most commonly used, however several other can be found in the literature (*e.g.* Hardin & Hilbe, 2012).

A GsLM is an extension of the GLM concept. Further extensions are the 'generalised estimating equations' (GEE), a GsLM for random effects; and the 'generalised mixed methods models' (GLMM), a GsLM for a mixture of random and fixed effects, also known

[1] Note the difference in terminology between general and generalised

as multilevel models. Further extensions and statistical approaches can be found in the literature (*e.g.* Myers *et al*., 2010).

Examples of literature where all the different aspects of experimental design are discussed at advanced level and in detail are Mead (1988), Altman (1991) and Quinn & Keough (2002).

St-Pierre (2007) reviewed the design and analysis of experiments using mixed methods in animal sciences. Experiments can be very useful to test aspects of husbandry in relation to welfare: *e.g*. bedding materials, housing designs, stocking rates, enrichments etc. Examples of studies using a trial design are Bilgili *et al*. (2009), Van de Perre *et al*. (2011) and Mancera *et al*. (2014). Bilgili *et al*. (2009) used randomised trials to study the influence of bedding materials on footpad dermatitis in broilers. Van de Perre *et al*. (2011) investigated pig behaviour in dependency of enrichments and weight. Mancera *et al*. (2014) used a Latin square design to study the effects of transport on eastern blue tongued lizards.

Mata and Mwakifuna (2012) is an experiment where 3 different breeds of chicken (Rhode Island Red, Black Australorp and the cross between these known as Hyblack) were used in two different production systems in Malawi (farm and scavenging). Survivability to disease and to predation (in the scavenging system only) were evaluated function of breed and production system as factors. Eggshell strength was used as a covariate in both models. GsLMs with a binomial link (complementary log-log) were successfully fitted differentiating breeds and eggshell strength as parameters affecting survivability.

Williams, Parrot and Da Mata (2012) is an experiment to evaluate levels of stress with the use of different horse dental instruments. A repeated measures ANOVA was applied as there were multiple measures made, using the different instruments repeatedly in the same horses.

2.5. Cross-sectional studies

Cross-sectional studies are surveys where a sample of individuals is selected within a defined population at a specific point in time. They are snapshots of the state of a particular population (species, breed, production system, husbandry practice, subgroup, wild population, etc.) in a particular space (local, region, country, etc.) in relation to a particular variable of interest (presence or absence of disease, trauma, dysfunction, behaviour, physiologic process, etc.), and in a defined interval of time (Last, 2001; Thrusfield, 2005; Aschengrau & Seage, 2008).

The first step in a cross-sectional study is the definition of the target population or universe of study. This population can be a relatively small group of local individuals or a large global population. The inference promoted by the study is limited by the universe considered: if a local sample is used, conclusions cannot be drawn for a wider population.

Power analysis for determination of sample sizes is the second step. With a larger sample sizes there is an increase in the power of the analysis, which is also in dependency of the critical significance level considered; higher levels of significance require larger samples and have higher power. In the opposite direction is the spread of the data; larger standard deviation requires a larger sample size for the same level of significance. The power varies also with the type of test to be performed, and therefore with the type of data, with parametric tests being more powerful than non-parametric. Cohen (1988) is the summit of work developed in the area by the author. Hawkins, Gallacher and Gammell (2013) reviewed the topic, and Fault *et al.* (2007) as well as Fault *et al.* (2009) makes a more comprehensive review of power analysis, introducing the G Power software at the same time. The 'G*Power 3' is a freeware software for 'Windows' and 'Mac' available with manuals from http://www.gpower.hhu.de/.

The third step is the sampling design: samples are ideally taken at random (probabilistically), ensuring the selection of a group of individuals, representative of the universe being targeted. Research constraints may, sometimes, determine non-probabilistic sampling methods; and convenience sampling is used to take advantage of circumstances allowing collection of data that could not be available at random: *e.g.* animals that are slaughtered, harvested, culled, may provide unique non-invasive opportunities to collect data otherwise difficult or impossible to collect. Non-probability sampling may be a source of bias and introduces limitations in the study. Probabilistic sampling should therefore be preferred; these include a variety of designs to allow the collection of representative groups of individuals: simple random, cluster, stratified, systematic, etc. A full explanation of methods, advantages and disadvantages of different probabilistic designs can be explored in the literature (*e.g.* Lohr, 2010).

After data collection, the last step is the analysis. In a first instance measures of prevalence and incidence are calculated (Table 4). Prevalence can be defined as the number of animals in the sample with a positive identification of the characteristic of interest, divided by the total number of animals in the sample. Incidence measures the proportion of new animals with the characteristic of interest in the given sample. Cross-sectional studies take place in a particular time and therefore temporal sequences of causes cannot be determined (Thrusfield, 2005; Aschengrau & Seage, 2008; Pfeiffer, 2010).

Cross-sectional studies identify the presence or absence of variables of interest in individuals, allowing the study of relationships with particular characteristics of the individuals. These relationships are known as 'risk factors': incidence and prevalence of the variable of interest in relation to 'exposure' or presence of characteristics in individuals (Pfeiffer, 2010).

The data collected may be nominal, in the form of counts (*e.g.* number of animals with the variable of interest, exposed, or treated) or dichotomic (*e.g.* presence or absence of characteristic, dead or alive, male or female). Data can also be collected in other formats, such as scale (measurements) or nominal in the form of ranks. Nominal variables are analysed using tables of contingency (Table 4), using chi square type statistics; using GsLM from the Poisson family (for counts) or binomial family (for dichotomic variables); or using non-parametric approaches when the data is presented as ranks, such as the Kruskal-Wallis for independent data and the Friedman test for repeated measures.

Tables of contingency are used to calculate standardised units (*e.g.* odds ratio, risk ratio) to measure an event (*e.g.* disease or any other characteristic of interest in animal welfare science). These are standardised measures independent of sampling numbers and therefore allowing comparisons of different studies. Table 4 details calculations of these measures.

Table 4 – The contingency table of observational studies and respective measures. A, B, C, D will represent the number of animals in each of the categories (Thrusfield, 2005).

	Condition present	Condition absent	Total
Postulated RF present (exposed animals)	A	B	A + B
Postulated RF absent (unexposed animals)	C	D	C + D
Total	A + C	B + D	A + B + C + D = N

In cross-sectional studies only N is predetermined

In cohort studies (A + B) and (C + D) are predetermined

In case-control studies (A + C) and (B + D) are predetermined

<u>In cross-sectional studies</u>

Prevalence odds ratio = A D / B C

<u>In cohort studies</u>

Probability condition present in exposed (Incidence for exposed) = A / (A + B)

Probability condition absent in exposed = B / (A + B)

Probability condition present for unexposed (Incidence for unexposed) = C / (C + D)

Probability condition absent for unexposed = D / (C + D)

Odds rate of condition, present, exposed (risk rate) = {A / (A + B)} / {B / (A + B)} = A / B

Odds rate of condition, present, unexposed = {C / (C + D)} / {D / (C + D)} = C / D

Condition odds ratio = (A / B) / (C / D) = A D / B C

<u>In case-control studies</u>

Probability of exposure with the condition = A / (A + C)

Probability of no exposure with the condition = C / (A + C)

Probability of exposure for controls = B / (B + D)

Probability of no exposure for controls = D / (B + D)

Odds rate of exposure (condition) present, exposed = {A / (A + C)} / {C / (A + C)} = A / C

Odds rate of exposure (control) absent, exposed = {B / (B + D)} / {D / (B + D)} = B / D

Exposure odds ratio (or risk ratio) = (A / C) / (B / D) = A D / B C

Note: RF – Risk Factors

Examples of cross-sectional studies in animal welfare science are Fatjó, Ruiz-de-la-Torre and Maneca (2006); and Ponzio *et al*. (2009). Fatjó, Ruiz-de-la-Torre and Maneca (2006) used a questionnaire directed to veterinary surgeries to assess the incidence of dogs and cats' behavioural problems. Ponzio *et al*. (2009) investigated the incidence of fur-chewing in commercial Argentinian chinchilla farms.

Mata and Lam (2013) is a cross-sectional study evaluating the parasite burden in birds of prey. A GsLM with a Poisson link (negative binomial) for counts was adjusted, differentiating parasite burden in dependency of risk factors (species of bird and diet type).

Mata, Johnson and Bishop (2015) is a cross-sectional study to investigate three different types of bit injuries in horses. A score was given to the degree of injury and its prevalence and severity was determined in function of the discipline (polo or race horse) and/or bit (gag or snaffle). As polo horses normally use gag bits and race horses snaffle bits, the conclusions cannot be made individually for discipline or type of bit. A Poisson GsLM (log) model was successfully fitted to the data associating discipline/bit with type of injury, in dependency of age/time in the sport which also entered in the model as an independent variable.

2.6. Cohort studies

Cohort studies are also known as prospective studies as from the perspective of the researcher they are set in the present to collect data in the future. In these type of studies the sample of the defined population is divided in segments (*e.g.* exposed, not exposed, and eventually considering different degrees of exposure) that are followed up to investigate the probability of development of conditions of interest. Normally a large number of individuals is involved in the study, especially if the event of interest is rare. The period of time depends on the life cycle of the individuals and the characteristic of interest.

These studies allow the comparison of incidence and prevalence over certain periods or points in time (Thrusfield, 2005). Comparisons between segmented groups of individuals within the defined population can be established using odd ratios or risk ratios: *e.g.* incidence rate in males / incidence rate in females. In this case cohort studies are analysed using tables of contingency (Table 4).

Again we may be in the presence of nominal, dichotomic and scale variables and the analytic models to be used are those mentioned in the previous sections. The novelty is the presence of the variable ‘time’, treated with a very specific type of model used in ‘survival’ analysis.

Survival analysis or ‘time to event’ statistics introduce the concept of ‘censored’ data. We may lose track of certain animals initially included in the study (*e.g.* lost to follow up in surgeries, sold by owners, owners moving, study ending before the event); if the event of interest still did not occur when the animal was last seen, we don’t know exactly when it occurred or will occur, but we know that at that particular point in time (when it was last seen) did not occur. The time registered for such animals is measured up to the last time seen and is entered in the analysis censored. The models used in survival analysis allow censored animals to be included in the study, and we can therefore, make use of this incomplete but precious information.

If the event of interest is death the term survival is appropriate, but not otherwise (*e.g.* time to develop a condition). In any case, in time to event analysis, models are used to calculate the probabilities of future events. The complementary probabilities add to the unit, *i.e.* if the probability of a dog dying two years after cancer diagnosis is 0.6, the probability of being alive is 0.4.

There are three main models to use depending on the data collected. If time is given grouped (*e.g.* 0-6 month, >6-12 month, >12-18 month, >18-24 month, >24 month) ‘life tables’ are used, otherwise we can use the Kaplan-Meyer or the Cox regression techniques. The Kaplan-Meyer technique is used for the analysis of factors of interest (*e.g.* breed, gender, exposure, housing system, etc.), allowing the identification of eventual significant differences in time to event probability for the different levels of that factor. The technique does not allow factorial design and therefore each factor is analysed individually. Finally the most flexible of the models is the Cox regression, allowing the inclusion of factorial designs and covariates (*e.g.* age at diagnosis, weight, height, etc.).

Examples of cohort studies in animal welfare sciences are Diesel, Pfeiffer and Brodbelt (2008), Machado *et al*. (2010) and Müller *et al*. (2010). Diesel, Pfeiffer and Brodbelt (2008) used a well-designed cohort study to identify risk factors affecting the success of rehoming dogs. Machado *et al*. (2010) studied the effect of claw horn disruption lesions and body condition score on survivability (time to death or culling) and on reproductive performance (time to conceive) of dairy cows. Müller *et al*. (2010) while studying the effect of captivity on longevity of three different species of deer, used life tables.

Mata (2014b) is a cohort study where a survival analysis in dogs diagnosed with appendicular cancer was conducted. The risk factors analysed were behaviour of the dog in the first week after amputation, gender, type of treatment, age at diagnosis, castration and type of cancer. A Cox regression was successfully fitted using dog behaviour and type of treatment as dependent variables. Chi-squared type statistics were also used to analyse the other variables.

2.7. Case-control studies

Case-control studies are also known as retrospective studies as from the perspective of the researcher they are set in the present to collect data from the past. Individuals are chosen in result of the presence or not of a characteristic of interest (disease, trauma, dysfunction, behaviour, etc.). Individuals with the characteristic are the cases and those without are the controls. The past of the individuals is then investigated to identify and differentiate degrees of exposure of postulated risk factors triggering the development of the characteristic of interest. The number of individuals to recruit to the study is smaller than in cohort studies, as the characteristic of interest, even if rare, is already diagnosed. Data in case-control studies is analysed with tables of contingency (Table 4).

Examples of case-control studies in animal welfare sciences are Lund, Agger and Vestergaard (1996) and Wylie *et al*. (2013). Lund, Agger and Vestergaard (1996) investigated reported behavioural problems of dogs at surgeries in Denmark. Wylie *et al*. (2013) investigated risk factors (turnout, stabling, feeding, transport, exercise, farriery and health) for equine laminitis.

In **Mata (2013)** a meta-analysis of case-control trials of vaccines against mastitis in dairy cattle was conducted. In the different studies used in this meta-analysis cows developing the condition are the cases and those that do not are the controls. They are then retrospectively investigated for identification of vaccination or not, which allows the production of exposure odd ratios for determination of vaccine efficacy. The outcome measure considered in the analysis was the logarithm of the risk ratio (see Table 4 for definition of risk ratio). As none of the moderators initially considered was found to be significant, a random effects model was adjusted to add for the heterogeneity observed. The model was also adjusted for the publication bias, once this was found significant.

Mata (2015) is another example of a case-control study where a GEE was successfully fit, to allow the calculation of probabilities of tooth health scores in cats at different ages and diet types. A GEE was used due to the presence of repeated measures in the data, as type of teeth were measured several times in the same individual (incisors, canines, premolars and molars), which therefore entered the analysis as a random factor. The different diets and age groups made the case and control for each other. The different cats entered in the study were scored for their teeth health status, which was then retrospectively analysed as a dependent variable. The independent variables in the study included the factors ‘type of teeth’ and ‘type of diet’, and the covariate ‘age’. This study is an example of the importance of choosing the correct statistical models, with inclusion of interactions between variables. The exclusion of interactions assuming independence between variables is a common pitfall in animal welfare risk assessment (Collins, 2012). The variable age was not found significant on its own, but was then found significant when entered in the model in interaction with type of teeth and diet.

2.8. Ecological studies

The previous studies have considered single individuals as the unit of analysis within a defined population; in ecological studies the unit of analysis is collective, *e.g.* farm, zoo, park, flock. Again the universe of study needs to be defined, *e.g.* farms in a region or country.

Ecological studies should be interpreted carefully, as the aggregated unit of analysis may introduce bias in the inferential process, leading to the so called ‘ecological fallacy’. The ecological fallacy is defined as “the bias that may occur because an association observed between variables on an aggregate level does not necessarily represent the association that exists at an individual level” (Porta, 2008 p.51); Piantadosi, Byar and Green (1988) and Greenland and Morgenstern (1989) discuss it in detail.

Ecological studies are used with advantage to identify geographical patterns of risk factors, and the ecological fallacy becomes an eventual problem only when risk factors are associated with other geographic patterns, *e.g.* related with environment, sociology or demography (Wakefield, 2008). The most common type of ecological studies are cross-sectional, without dependency of time; longitudinal ecological studies can, however also be performed. The consideration made previously for observation studies in terms of analytical procedures apply, therefore to ecological studies.

Examples of ecological studies are Moinard *et al.* (2003) and Alvåsen *et al*. (2012). Moinard *et al.* (2003) in a case-control ecologic study, investigated risk factors (several including aspects of management, housing, hygiene and production systems) for the presence of tail biting in pigs, using farm as the unit of analysis. Alvåsen *et al*. (2012) in a case-control ecological study, investigated risk factors (average milk yield, average herd size, predominant breed, average calving interval, season management type housing system and region) associated with cow mortality in Swedish dairy herds (units of analysis).

Mata, Williams and Marks (2012) is a case-control ecological study as the unit of analysis is the race including several horses. The study aimed to identify risk factors for horses being pulled up from races at the Cheltenham racetrack. The risk factors investigated were the number of racers, distance of the race, conditions of the track and the number of fences. A GsLM with a binomial link (logit) is used to model the probability of existence of pulled up horses and a GsLM with a Poisson link (negative binomial) to model the number of pulled up horses per race.

2.9. Conclusion

The different epidemiological study designs were explored and put in an animal welfare perspective. Each of the study designs was illustrated with, at least, one of the publications part of this thesis. The methodology used to produce these papers was therefore herein discussed and justified. This chapter concludes therefore the first objective of this thesis: “To explore the different epidemiological study designs and biostatistical approaches and put them in an animal welfare scientific context, developing a coherent framework of useful standard techniques”.

Chapter 3: Appraisal of Publications

3.1. Introduction

In the previous chapter the methodology used in the papers part of this thesis was discussed and justified. In this chapter the results obtained in those papers are appraised with a focus on a discussion of their contribution to knowledge, which also highlights the advantages with the use of epidemiology in animal welfare science (section 3.5). The comments on the standing of the journals in which the papers were published and on their public reception as indicated by their citations is made in section 3.2. In Section 3.3 the acknowledgement of the contribution I have made in each of the papers is outlined and in section 3.4 the choice of the papers is justified with the presentation of a matrix showing the interrelationship between them.

3.2. Journal standings and public reception

From the nine publications submitted with this thesis, and accordingly to the standings of the journals where they were published there are six of higher quality indexed on SCOPUS from which five are indexed on the ISI Web of Science. The six SCOPUS indexed journals stand in the second quartile of the respective subject (veterinary science, equine science and animal science & zoology). The five ISI Web of Science indexed journals stand one in the second quartile (agriculture, dairy and animal science) and three in the third quartile (veterinary science); the fifth journal does not contain this metric. The SCOPUS metrics are relatively more generous showing six out of the total nine publications above the median of all the research in their respective subject areas. Complete information is fully available in Tables 5 – 13.

Despite all the nine publications being very recent (there is one publication from 2012, two from 2013, two from 2014 and two from 2015), there are already eight citations in regular academic journals (Table 14). There are also references to the publications in lay press online and paper magazines (Table 15).

Table 5 – ‘‘The Veterinary Nurse’ journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

The Veterinary Nurse

Publisher: M.A. Healthcare, Ltd.

SJR: N/A

TR Impact Factor: N/A

Indexed: CrossRef, Google Scholar

Table 6 – ‘Animals’ journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher: Multidisciplinary Digital Publishing Institute

SJR: 0.29; Q2 (Veterinary Sciences)

TR Impact Factor: N/A

Indexed: AGORA (FAO) AGRIS (FAO), AGRICOLA (NAL), CAB Abstracts (CABI), DOAJ, PubMed (NLM), SCOPUS (Elsevier), Zoological Record (TR), Web of Science (TR)

Table 7 – ‘Comparative Exercise Physiology’ journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher : Wageningen Academic Publishers

SJR: N/A

TR Impact Factor: N/A

Indexed: AGRICOLA (NAL), CAB Abstracts (CABI), Global Health (CABI), Nutrition and Food Sciences Database (CABI), VetMed Resource (CABI)

Table 8 – 'British Poultry Science' journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher: Taylor & Francis, Ltd

SJR: 0.52; Q2 (Animal Science & Zoology)

TR Impact Factor: 0.936; ISI JCRR: Q2 (Agriculture, Dairy and Animal Science)

Indexed: AGRICOLA (NAL), Agricultural Engineering Abstracts (CABI), Animal Breeding Abstracts (CABI), BIOSIS Previews (TR), Chemical Abstract Services (ACS), Current Contents/Agriculture, Biology & Environmental Sciences (TR), Food Science and Technology Abstracts (CABI), Helminthological Abstracts (CABI), Index Veterinarius (Global Health), MEDLINE/PubMed (NLM), Nutrition Abstracts and Reviews (CABI), Poultry Abstracts (CABI), Research Alert (EBSCOhost), Science Citation Index (TR), SciSearch (DMDI), SCOPUS (Elsevier), Veterinary Bulletin (CABI), Wildlife Review Abstracts (CABI) and World Agricultural Economics & Rural Sociology Abstracts (CABI)

Table 9 – 'Journal of Applied Animal Welfare Science' journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher: Taylor & Francis, Ltd

SJR: 0.48; Q2 (Veterinary Sciences)

TR Impact Factor: 0.739; ISI JCRR: Q3 (Veterinary Sciences)

Indexation: CAB Abstracts (CABI), Chemical Abstracts Service (CSA), AGRICOLA (CSA), PsycINFO (CSA), Academic Search Alumni Edition (EBSCOhost), Academic Search Complete (EBSCOhost), Academic Search Premier (EBSCOhost), Biological Abstracts (EBSCOhost), Current Abstracts (EBSCOhost), TOC Premier (EBSCOhost), Wildlife & Ecology Studies Worldwide (EBSCOhost), EMBASE (Elsevier), SCOPUS (Elsevier), PubMed (NLM), Biological Abstracts (TR), BIO SIS Previews (TR), Current Contents (TR), Social Sciences Citation Index (TR), Web of Science (TR), Zoological Record Online (TR), OCLC, ProQuest, Wildlife Review Abstracts, SwetsWise

Table 10 – 'Journal of Equine Veterinary Science' journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher: W.B. Saunders Ltd

SJR: 0.34; Q2 (Equine Science)

TR Impact Factor: 0.871; ISI JCRR: Q3 (Veterinary Sciences)

Indexed: SCOPUS (Elsevier), BIOBASE (Elsevier), AGRICOLA (NAL), Current Contents/Agriculture & Biology & Environmental Sciences, Science Citation Index, Index Veterinarius (Global Health I), Veterinary Bulletin (Global Health), CAB Abstracts (CABI)

Table 11 – 'Journal of Veterinary Behavior: Clinical applications and research' journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2015) and indexation databases.

Publisher: Elsevier

SJR: 0.52; Q2 (Veterinary Sciences)

TR Impact Factor: 0.957

Indexed: Index Veterinarius (Global Health), Veterinary Bulletin (Global Health), CAB Abstracts (CABI), PsycINFO (APA), SCOPUS (Elsevier), Science Citation Index Expanded (TR), Journal Citation Reports (TR)

Table 12 – 'Revista Portuguesa de Ciências Veterinárias' journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher: Sociedade Portuguesa de Ciências Veterinárias

SJR: N/A

TR Impact Factor: N/A

Indexed: Biblioteca Digital Vérsila (Open Archives Initiative), Index Revistas Médicas Portuguesas, Latindex, CAB Abstracts (CABI), AGRIS (FAO), Google Scholar

Table 13 – 'Zoo Biology' journal standings. Identification of the publisher, SJR SCImargo Scientific Journal Rankings with; quartile placement in subject area (2014), TR Thomson Reuters Impact Factor with ISI JCR Journal Citation Report quartile placement in subject area (2014) and indexation databases.

Publisher: John Wiley & Sons, Inc.

SJR: 0.45; Q2 for Animal Science & Zoology Journals

TR Impact Factor: 0.831; ISI JCRR: Q3 (Veterinary Sciences); Q3 (Zoology)

Indexed: Abstracts on Hygiene & Communicable Diseases (CABI), AgBiotech News & Information (CABI), AgBiotechNet (CABI), Agricultural Engineering Abstracts (CABI), Agroforestry Abstracts (CABI), Animal Breeding Abstracts (CABI), Biocontrol News & Information (CABI), CAB Abstracts (CABI), CABDirect (CABI), Dairy Science Abstracts (CABI), Forest Products Abstracts (CABI), Forestry Abstracts (CABI), Grasslands & Forage Abstracts (CABI), Helminthological Abstracts (CABI), Horticultural Science Abstracts (CABI), Index Veterinarius (CABI), Leisure Tourism Database (CABI), Leisure, Recreation & Tourism Abstracts (CABI), Maize Abstracts (CABI), Nutrition Abstracts & Reviews Series B: Livestock Feeds & Feeding (CABI), Pig News & Information (CABI), Plant Genetic Resources Abstracts (CABI), Postharvest News & Information (CABI), Poultry Abstracts (CABI), Review of Agricultural Entomology (CABI), Review of Medical & Veterinary Entomology (CABI), Rural Development Abstracts (CABI), Soils & Fertilizers Abstracts (CABI), Soybean Abstracts Online (CABI), Sugar Industry Abstracts (CABI), Tropical Diseases Bulletin (CABI), , Wheat, Barley & Triticale Abstracts (CABI), World Agricultural Economics & Rural Sociology Abstracts (CABI), Veterinary Bulletin (Global Health), AGRICOLA (NAL), BIOBASE (Elsevier), Biological Abstracts (TR), BIOSIS Previews (TR), Chemical Abstracts Service (ACS) Animal Behavior Abstracts (CSA, ProQuest), Biological Sciences Database (CSA, ProQuest), Environmental Sciences & Pollution Management Database (CSA, ProQuest), Human Population & Natural Resource Management (CSA, ProQuest), Current Contents: Agriculture, Biology & Environmental Sciences (TR), Ecology Abstracts (CSA, ProQuest), Journal Citation Reports/Science Edition (TR), MEDLINE/PubMed (NLM), PsycINFO/Psychological Abstracts (APA), PubMed Dietary Supplement Subset (NLM), Science Citation Index (TR), SCOPUS (Elsevier), VINITI (All-Russian Institute of Science & Technological Information), Web of Science (TR), Zoological Record (TR)

Table 14 – Known citations in academic journals of the nine publications part of this thesis. Source of information: [1] SCOPUS, [2] Web of Science, [3] Google Scholar

Mata and Mwakifuna (2012)

[1,2,3] Chikumba, N., Chimonyo, M., Mapiye, C., Dugan, M.E.R. (2014) Physicochemical properties of breast meat from water-stressed naked-neck and Ovambo chickens. *British Poultry Science*. 55 (2), pp.197-206.

[1,3] Munisi, W. G., Mbaga, S. H., Katule, A. M. (2015) Evaluation of growth characteristics of parental, F, F 2 and backcross chickens from broiler and Black Australorp stocks in Tanzania. *Livestock Research for Rural Development*. 27 (12), article# 235.

[1] Ncobela, N., Chimonyo, M. (2016) Nutritional quality and amino acid composition of diets consumed by scavenging hens and cocks across seasons. *Tropical Animal Health and Production*. 48 (4), pp.769-777.

[3] Munisi, W. G., Katule, A. M., Mbaga, S. H., (2016) Comparisons of egg production and quality traits of parental and crosses of broiler and Black Australorp chickens in Tanzania. *Livestock Research for Rural Development*. 28 (6), article# 111.

Mata, Williams and Marks (2012)

[*,3] Williams, J. M., Marlin, D. M., Langley, N., Parkin, T. D., Randle, H. (2013) The Grand National: a review of factors associated with non-completion and horse-falls, 1990 to 2012. *Comparative Exercise Physiology*, 9 (3-4), pp.131-146.

[1,3] Wilk, I., Janczarek, I, Zastrżynńska, M. (2016) Assessing the suitability of thoroughbred horses for equestrian sport after their racing careers. Journal of Veterinary Behavior: Clinical Applications and Research, 15(5), pp.43-49.

Williams, Parrot and Da Mata (2012)

[1,2] Tremaine, H. (2013) Advances in the treatment of diseased equine cheek teeth. *Veterinary Clinics of North America: Equine Practice.* 29 (2), pp.441-465.

[1,2] Overall, K. L. (2012) What horses can teach dogs about welfare and ethics. *Journal of Veterinary Behavior: Clinical Applications and Research.* 7 (3), pp.119-122.

Mata (2014a)

[3] Noleto, P.G., Cubas, J.P.C., Barbosa, F.C., Guimarães, E.C., Mundim, A.V. (2016) Biochemical profile of polo horses in training phase and those players of official competition. *Comparative Clinical Pathology*. 25 (4), pp.911-915.

Mata, Johnson and Bishop (2017)

[1,3] Borstel, U.K., Visser, E.K., Hall, C. (*in press*) Indicators of stress in equitation. Applied Animal Behaviour Science, 190 (5), pp.43-56.

* Self-citation by one of my co-authors

Table 15 – Known citations in lay press magazines of the nine publications part of this thesis.

Mata (2015)
Anon (2015) Bad breath. Go Pets America, [online] Available at: <http://www.gopetsamerica.com/cat-health/bad_breath.aspx> [Accessed 14 June 2016]
Mata, F., Johnson, C. and Bishop, C. (2015)
Oke, S. (2015) Researchers study bit-induced trauma. *The Horse: your guide to equine health care,* [online] Available at: <http://www.thehorse.com/articles/35500/researchers-study-bit-induced-trauma> [Accessed 14 June 2016]
Anon (2015) Researchers probe bit-related injuries in racehorses and polo ponies. *horsetalk.co.nz,* [online] Available at: < http://www.horsetalk.co.nz/2015/04/20/bit-related-injuries-racehorses-polo-ponies/#axzz4ENLnS5nm> [Accessed 14 June 2016]
Anon (2015) Racehorses in snaffle bits more likely to experience oral trauma than polo ponies in gag bits. *HorseScience.co,* [online] Available at: <http://horsescience.co/2015/03/racehorses-in-snaffle-bits-more-likely-to-experience-oral-injuries-than-polo-ponies-in-gag-bits/> [Accessed 14 June 2016]

3.3. Acknowledgement of contributions in the publications part of this thesis

Considering an agreed contribution in co-authored papers, I am claiming the authorship of the equivalent to seven full papers. Table 16 summarises these calculations. I am the first author in eight of the nine papers submitted with this thesis, from which four are single authored, two papers have two authors and two papers three authors. I am the third author in one paper with three authors. Detailed information regarding the contribution of each one of the authors can be found in the statement that indicates the extent of the contribution by other collaborating researchers with reference to the contribution to design, analysis, conduct of the research and writing up of the publication, found in appendix II.

Table 16 – Research outputs part of the present thesis and acknowledgement of my contributions with the calculation of the overall full paper equivalent.

Paper	No. of authors	Weighted contribution
Mata & Mwakifuna (2012)	2	0.8
Mata, Williams and Marks (2012)	3	0.5
Mata (2013)	1	1
Mata & Lam (2013)	2	0.8
Mata (2014a)	1	1
Mata (2014b)	1	1
Mata (2015)	1	1
Mata, Johnson and Bishop (2015)	3	0.7
Williams, Parrot and Da Mata (2012)	3	0.2
Full papers equivalent		7

3.4. Thematic and methodological interrelationships among the publications

In the second chapter I have explored different methodologies used in epidemiology and have illustrated these with the papers part of the portfolio submitted with this thesis. While using different epidemiological approaches I have also used a variety of biostatistical approaches also applied to a variety of animal settings. I have therefore applied a framework of useful standard techniques that integrate animal welfare and epidemiology.

Accordingly to the matrix presented in Table 17, I have covered in the portfolio of nine publications submitted with this thesis, the full range of epidemiological study design. I have also covered all the animal welfare assessment clusters considered by Fraser (2009): physical health and functioning, affective states (distress, fear, pain) and ability to live natural lives. A third dimension of analysis in the matrix presented in Table 17 includes the range of bio-statistical approaches used. Finally the fourth dimension of this matrix includes a range of animal settings: farm, equine, companion and captive. Table 17 is intended to provide an overview of the methodological interrelationship among the publications. It shows how the publications part of this thesis covered a range of possible research aspects produced by integrating animal welfare and epidemiology.

Table 17 – Matrix showing the interrelation between the papers and placing the different papers accordingly to the dimensions considered: type of epidemiological study (as per Figure 2) and area of animal welfare assessed (clusters considered by Fraser, 2009). The biostatistical method used in the analysis (Italic) and the type of animal setting (bold) are also identified.

Animal Welfare / Epidemiology	Physical health and functioning	Affective states: distress, fear, pain	Ability to live natural lives
Experiment		Williams, Parrot & Da Mata (2012) *RM ANOVA* **Equine**	Mata & Mwakifuna (2012) *GsLM (c log-log)* **Farm (poultry)**
Cross-sectional	Mata & Lam (2013) *GsLM (neg. binomial)* **Captive (raptors)**	Mata, Johnson & Bishop (2015) *GsLM (log)* **Equine**	
Case-control	Mata (2015) *GEE* **Companion (cat)**	Mata (2013) *Meta-analysis* **Farm (dairy cattle)**	
Cohort		Mata (2014b) *Survival analysis* **Companion (dog)**	
Ecological	Mata, Williams and Marks (2012) *GsLM (logit)* *GsLM (neg. binomial)* **Equine**		
Model	Mata (2014a) *Mathematical model* **Equine**		

Note: RM ANOVA – repeated measures analysis of variance; GsLM – generalised linear models, GEE – Generalised estimating equations

3.5. The advantageous use of epidemiology in research and contribution to knowledge in animal welfare science

Epidemiology can be used with advantage in the study of animal welfare (Willeberg, 1991, 1997; Waiblinger, Knierim & Winckler, 2001; Rushen, 2003; Green & Nicol, 2004; Broom, 2006; Barber, 2009; Millman *et al.*, 2009; Collins *et al*., 2010; Collins, 2012; Collins & Part, 2013; Paton, Martin & Fisher, 2013; and Mendl *et al*., 2016).

Epidemiology is useful in animal welfare in the identification of risk factors behind the development of maladaptation and abnormal behaviours (Green & Nicol, 2004) or more broadly animal welfare hazards (Barber, 2009; Collins *et al.*, 2010). Animal welfare scientists have relied excessively on experimental approaches in controlled laboratory environments in an attempt to control confounders (Rushen, 2003). Animal environment systems are complex and have a multivariate nature (*e.g.* animal interactions, nutrition, and climate) (Waiblinger, Knierim & Winckler, 2001; Rushen, 2003). Therefore a holistic approach, such as epidemiology, which considers multiple interacting variables, is more effective in capturing factors responsible for poor welfare (Waiblinger, Knierim & Winckler, 2001; Rushen, 2003).

In **Mata and Mwakifuna (2012)**, our research aim was to determine factors affecting health and welfare, functioning, affective states and ultimately behaviour, which could impact on survivability by resistance and avoidance of predators in chicken. A negative correlation between egg shell quality and survivability, never reported before, was found. The genetic traits of modern breeds of chicken selected for enhanced egg production were discussed as a potential cause for the problem. Modern breeds with superior egg traits become depleted of calcium which impairs their foraging and ability to escape predators, limiting their survivability when returned to production systems closer to their original environments (in this study the Malawi scavenging production systems used by natives in their villages). In the study a maladaptation through a change in behaviour in Black Australorp birds was also reported. These birds show a continuous broody behaviour, nesting in bare soil and therefore increasing susceptibility to predation.

Highly selected chicken breeds show a maladaptation to scavenging production systems in tropical and rural Malawi, despite the excellent productive performance as layers in

European and North American industrial production systems. Eggshell strength is a trait positively selected in these breeds and may not be identified as a risk factor in industrial production systems, where designed rations are offered to the birds, but that is not the case in traditional production systems.

The source of risk factors varies from genetic and environment such as in **Mata and Mwakifuna (2012)** to husbandry and management (including nutrition, health, housing and others). In **Mata and Lam (2013)** and **Mata (2015)**, feeds and types of feed were associated respectively with increased burdens of parasites in birds of prey and tooth health in cats.

Studies of endo-parasitology in captured and reintroduced birds of prey are rare (Sanmartin *et al*., 2004; Santoro *et al.*, 2010). In **Mata and Lam (2013)** we established an original relationship between diet, species and parasite burden: birds feeding on mice and whole chicken carcass were found to have an increase in the helminthic parasite burden, in contrast with a diet based in chicken breast and day chicks. The result was explained by the higher probability of the former diets containing contaminated offal, with a larger variety of helminthic fauna.

In **Mata (2015)** I concluded that dry food should be present in cats' diet from an early age to promote tooth abrasion. In contrast, wet canned food was found to contribute to an increased accumulation of calculus in tooth which is the first step in the development of periodontal disease. This conclusion is not original, however an original relationship was established between age of the cat and type of teeth, and it was found that cheek teeth are more susceptible to poor oral health independently of the age of the cat.

Multivariate methods used in epidemiology are advantageous in the identification of animal welfare risk factor interactions (Waiblinger, Knierim & Winckler, 2001), as per 'age' and 'tooth' in **Mata (2015)**. Epidemiology does not completely identify the underlying mechanisms conducting to the problem. However, the identification of the problem itself is highly valid even without an explanation for the reasons behind that fact (Green & Nicol, 2004; Barber, 2009). Sometimes a full explanation is not given but hypothesis are raised for further investigation, such as in **Williams, Parrot and Da Mata (2012)**, **Mata (2014b)** and **Mata, Johnson and Bishop (2015)**.

Williams, Parrot and Da Mata (2012) is an experiment to evaluate levels of stress with the use of different horse dental instruments. We found that motorised rasping of dental arcades promotes lower levels of stress when compared with manual rasping. This result contradicts the belief that the noise and action of motorised equipment may promote stress.

In **Mata (2014b)** factors such as gender, age and behaviour were associated with different risks for development of appendicular cancer in dogs, in the form of different survival times after limb amputation. An attempt to justify this differentiation is given, but these need to be confirmed by further research. Meanwhile the knowledge of the existence of predisposition factors is important on its own. The results confirm the beneficial effects of chemotherapy. The original result however is the relationship established between survivability and behaviour displayed in the first week after amputation. It is made evident that dogs displaying a more active behaviour are related with a longer life expectancy. With regards to gender it was found that bitches have higher probabilities of developing appendicular cancer than dogs, having the later a higher probability of being subject to limb amputation by traumatic causes rather than cancerous. It is hypothesised that higher frequencies of mammary cancer in bitches and a higher degree of aggressiveness in dogs, may explain these results, but further investigation is needed. Further results include castrated/spayed dogs being diagnosed for appendicular cancer later in life than intact dogs, and cancerous causes of amputation being diagnosed later in dogs' lives than traumatic causes.

Epidemiology allows the calculation of the prevalence of risk factors in particular populations (Collins *et al.*, 2010). In **Mata, Johnson and Bishop (2015)** we established an association between bit type/horse riding discipline and different types of oral trauma. The results obtained are original, as no similar studies were reported before. Race horses with snaffle bits were predisposed to significantly higher severities and prevalence of oral trauma than were polo ponies using gag bits. Only polo ponies were observed with tongue trauma. Race horses had higher severities of injuries in the commissures and bone spurs. Positive correlations were also established between age and / or time in sport and induced bit injuries.

Epidemiology also has the advantage of introducing the use of standardised procedures in animal welfare research by allowing comparisons and therefore decreasing the bias of individual studies (Croney & Millman, 2006). Also facilitates the integration for evidence synthesis in systematic reviews and meta-analysis (Barber, 2009).

The meta-analysis I have conducted in **Mata (2013)** made use of previous case-control studies done in a standardised manner allowing comparisons. Previous research is inconclusive regarding the benefits of vaccination against mastitis and the aim of this meta-analysis was to contribute to clarification of an eventual benefit of vaccination. The results show that vaccination can be advantageous, however it is concluded that the evidence produced does recommend the use vaccination to complement other biosecurity measures. Despite the conclusion that this vaccine does not solve the problem on its own, the welfare advantages are made evident.

All kinds of pathologies impact on animal welfare and are sometimes underused in animal welfare assessment (Houe, 2003; Rushen, 2003; Broom, 2006). Mastitis is a health problem but is the most important welfare issue in intensive dairy systems (Willeberg, 1997). The result of this meta-analysis is especially important in organic production systems where the use of antibiotics is limited and therefore any additional preventive measures are welcomed (von Borell & Sørensen, 2004).

Epidemiology allows animal welfare scientists to leave the relatively small scale of the experiment in a laboratory environment. Complex settings such as farms or zoos may be difficult to replicate in a laboratory, and observation studies are therefore more appropriate (Barber, 2003; Rushen, 2003; Green & Nicol, 2004; Millman *et al*., 2009). In more complex designs farms and zoos can even be the units of analysis (ecological studies) allowing the use of factors of analysis that differentiate between these units. **Mata, Williams and Marks (2012)** use horse races as the unit of analysis to investigate reasons for the existence of pulled up horses in a race. One previous study only was identified as having investigated this topic. This is therefore an area of research interest as horses are normally pulled up in races due to anomalies predictive of sub clinic or actual injuries. The knowledge of factors associated with the existence of pulled up horses can therefore benefit the welfare of racing horses. Factors such as the number of riders and the distance were associated with higher probabilities of existence of pulled up horses. The number of horses in the race and the race distance was positively associated with higher number of pulled up horses, which was identified before and again in this study. Faster runs, however have never been reported before as being associated with lower number of horses being pulled up. A larger number of horses and longer distances are obviously associated with lower speeds, but this is also the case if the horse is not fit for purpose.

If most of the advantages of the use of epidemiology in animal welfare are related with the identification of risk factors, there is however an exception. Mathematical modelling can also be used with advantage in risk assessment (Collins & Part, 2013). Semantic models take a description of a system with identified risk factors as input and produce a weighted welfare score as output (Bracke *et al.*, 2008, Collins, 2012; Paton, Martin and Fisher, 2013). Expeditious models may be used *in situ* with advantage, in welfare assessment of the physical state of the animals. This is exactly the case with the model I have developed in **Mata (2014a)**, which allows the prediction of the fitness degree in polo ponies. The model was used before in post-exercise heart rate recovery of humans, but was never fitted before to equine data. It is argued that the model has a high degree of accuracy and the potential to be used in simple portable equipment allowing quick *in situ* evaluation of horse fitness for exercise. The half recovery time has potential to be used as a measure of fitness degree, allowing comparison between horses.

This is a relevant contribution to knowledge, as the evaluation by heart rate recovery requires the standardisation of processes to allow comparison between horses. The linearization of the heart rate recovery model allows the analysis of variance and covariance, and therefore the future study of factors (such as breed, discipline and gender) and covariates (such as age and time or distance). These could then be used to calculate coefficients for standardization of measures, which would make comparison between horses possible. In disciplines such as endurance riding where ‘vet gates’ for horse fitness inspection are compulsory, this model could be applied with advantage.

3.6. Conclusion

The interrelationship between the publications part of this thesis was established, justifying their choice with the attempt to cover as much as possible the matrix displayed (Table 17), which is the essence of this thesis aim. The publications were appraised and the contribution to knowledge of each paper was identified to fulfil the second research objective of this thesis: “to identify, discuss and assess novel animal welfare risk factors in a full range of animal settings: production, captive, companion and equine”. Epidemiology is therefore proofed to be a very helpful tool to research animal welfare.

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Note: Full references in bold are the publications part of this thesis

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Appendix I: Publications One to Nine

Publication one

Mata, F. (2015) The choice of diet affects the oral health of the domestic cat. *Animals*. 5 (1), 101-109. DOI: 10.3390/ani5010101

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Article

The Choice of Diet Affects the Oral Health of the Domestic Cat

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Simple Summary: Oral health was assessed in different teeth of 41 cats of different ages and diets. It was found that oral health in cats varies with the variables considered. Incisors of young or adult cats, fed a dry diet, had better health in comparison to cheek teeth of older cats fed a wet diet. It is argued that cats' oral health may be promoted with an early-age cheek teeth hygiene and provision of abrasive dry food.

Abstract: In this cross-sectional study, the gingivitis and the calculus indices of the teeth of $N = 41$ cats were used to model oral health as a dependent variable using a Poisson regression. The independent variables used were "quadrant", "teeth type", "age", and "diet". Teeth type ($p < 0.001$) and diet ($p < 0.001$) were found to be significant, however, age was not ($p > 0.05$). Interactions were all significant: age x teeth ($p < 0.01$), age $\times$ diet ($p < 0.01$), teeth $\times$ diet ($p < 0.001$), and teeth $\times$ age $\times$ diet ($p < 0.001$). The probability of poor oral health is lower in the incisors of young or adult cats, fed a dry diet in comparison to the cheek teeth of older cats fed a wet diet. Diet has a higher contribution to poor oral health than age. It is argued that cats' oral health may be promoted with an early age hygiene of the cheek teeth and with provision of abrasive dry food.

Keywords: cat; diet; oral hygiene; periodontal disease; teeth

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1. Introduction

Periodontal disease (PD) has been recognised as one of the most prevalent diseases in cats, affecting around 70% of the domestic cats over two years of age [1], and 85% of those aged over five years [2]. PD is a generic term of a plaque-induced inflammatory condition, affecting the periodontium [3]. The aetiology of this condition starts with the formation of dental plaque that extends into the gingival sulcus and, with the aid of the calcium salts from saliva, produces the calculus, which is the main cause for the development of gingivitis [4]. Plaque is a layer of microorganisms, mostly bacteria, adhered to the teeth, and is responsible for the initiation of PD [5]. PD initially begins with gingivitis, which can then develop into periodontitis if left untreated. While gingivitis is reversible by treatment, PD of brachydont teeth is irreversible and can only be managed to avoid further progression, once irreversible destruction of connective tissues and loss of adjacent bone has taken place [4,6]. Systemic disease has been increasingly recognised in cats affected by PD [1,4]. Research suggests that PD has an association with the development of cardiorespiratory, hepatic, and renal disorders [4], and also diabetes mellitus in humans [7].

Calculus formation and the development of gingivitis are key aspects in the development of PD; as calculus and gingivitis increase, oral health deteriorates. A relationship between the degree of calculus and gingivitis development signals, therefore, a deterioration of the teeth health status. The calculus index (CI) proposed by Ramfjord [8], and the gingival index (GI) proposed by Loe and Silness [9] are still used today to assess the degree of development of these two conditions and can, therefore, be used to assess the oral health of cats. The GI scoring criteria are: 0 (normal), 1 (mild inflammation, slight colour change, slight oedema, no bleeding on palpation), 2 (moderate inflammation, redness, oedema, bleeding on probing), and 3 (severe inflammation, marked redness and oedema, tendency to spontaneous bleeding). The CI scoring criteria are: 0 (no calculus present), 1 (supra gingival calculus covering one third of the exposed tooth surface), 2 (supra gingival calculus covering more than one third to two thirds of the exposed tooth surface or presence of flecks of sub gingival calculus, or both), 3 (sub gingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of sub-gingival calculus around the crevices of teeth or both).

The prevalence and severity of PD varies with several factors, such as: gender, age, breed, diet, chewing behaviour, and systemic health [6]. Several studies have related the type of diet and age with the development of PD (e.g., [4,6,10]) but the relationship between these factors and the type of teeth has not yet been considered. Watson [4] performed a revision of the literature on the relationship between PD and diet in dogs and cats, and argues that the main advances in diet formulation for these animals have improved their health, especially in relation to nutritional deficiencies; on the other hand he points out the importance of the physical properties of the diet (texture, abrasiveness and chewiness) as additional methods to control plaque and prevent PD. Clarke and Cameron [6] compared the development of calculus and PD in domestic and feral cats in Australia, and found that calculus develops easily in domestic cats but no differences were found for PD. Once domestic cats were being fed with a canned and dry diet, they concluded that the live prey-based diet may prevent the development of calculus. Gawor *et al.* [10], while studying the influence of diet on oral health of dogs and cats, concluded that dental calculus and plaque were less frequent in cats fed dry, rather than wet, food. These authors also observed a positive correlation between age and calculus formation.

Previous studies considered the factors of age and type of diet individually, without looking into the type of teeth and into the interactive effects. The aim of this study is to verify the interactive effects of age, type of diet, and type of teeth, and to develop a stochastic model to allow the prediction of the impact on the oral health and welfare of cats.

2. Experimental Section

In this cross-sectional study, data were collected within Pet Doctors™ Veterinary Hospital on the Isle of Wight in England, taken from $N = 41$ Domestic Short Hair cats, during January 2013. The study did not require approval from an Ethics Committee, as it was based in data collected from normal clinical practice. Pet Doctors™ Veterinary Hospital is part of the Corporate Veterinary Surgeons (CVS UK) Limited and complies with the ethical rules and regulations on the treatment of animals set by the British legislation and professional bodies. All the cats used in the study are healthy animals, presented for surgery for other than the reasons of disease or illness: routine worming or check-up, spaying, or a simple post-traumatic procedure. All cats in this study had oral home care.

The gingivitis index (GI) and the calculus index (CI) were assessed for each of the cat's teeth, always by the same veterinary surgeon to avoid assessors' bias. The examination was done with the cat awake, without use of any medication or anaesthesia. None of the cats was subjected to a frequent type of dental hygiene. The cat owners were asked to fill in a questionnaire concerning their pet, having been explained the purpose of the questionnaire. Questions asked: "age" (young—up to 3 years, adult—from 3 to 8 years, and old—more than 8 years) and predominant "diet" (commercial dry, commercial wet, mixed commercial dry and wet, homemade).

Data were organised by quadrant ("side"—left or right, and "position"—maxilla or mandible) and "type of teeth" (incisors, canines, premolars, and molars). Teeth nomenclature used the TRIDAN modified system [11], according to Figure 1. Averages for CI and GI were calculated between: 101, 102, and 103 for upper right incisors; 201, 201, and 203 for upper left incisors; 301, 302, and 303 for lower left incisors; 401, 402, and 403 for lower right incisors; 106, 107, and 108 for upper right premolars; 206, 207, and 208 for upper left premolars; 307 and 308 for lower left premolars; and 407 and 408 for lower right premolars. For canines and molars, the values entered were those assessed as only one of these tooth types exist per quadrant. Finally, GI and CI were added together to create the variable teeth health status (THS). Values were rounded to the nearest unit and ranged from 0 to 6, as both CI and GI ranged from 0 to 3.

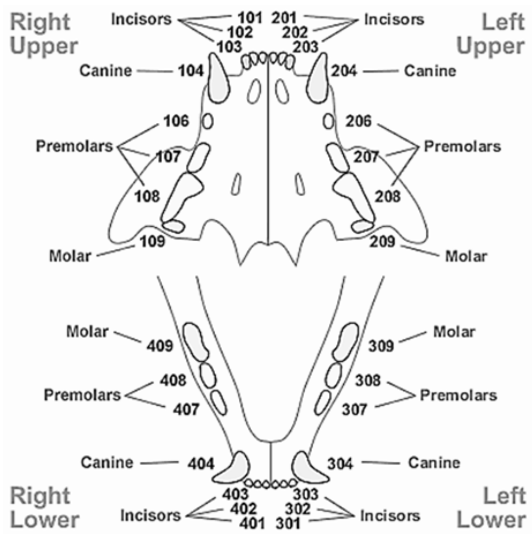

Figure 1. The TRIDAN modified system for cat teeth classification. Adapted from Crossley [12].

A generalised estimating equation approach was used to account for the within-subject effects of the variables side, position and type of teeth. A multinomial cumulative logit link function was fitted using THS as the dependent variable. The variables of age, type of teeth (incisive, canine, premolar, molar), and diet (dry, wet, mixed, homemade) were entered in the analysis as factors; first and second level interactions between factors were also entered. A type III sum of squares was chosen once the analysis had an unbalanced design. The variables were entered in the model following a forward stepwise procedure and were tested using the Wald Chi-square statistic, with the significance level set to $p < 0.05$. The analysis was done via generalised linear models routine, using the software IBM® SPSS® Statistics for Windows®, version 21.0. (IBM Corp., Armonk, NY, USA, 2012).

3. Results and Discussion

3.1. Results

From the factors analysed, teeth ($p < 0.001$) and diet ($p < 0.001$) were shown to be significant. Age was not significant ($p > 0.05$) as a stand-alone factor, however it was found significant within first and second level interactions, together with the 1st and 2nd order interactions between all the other factors: age × teeth ($p < 0.01$), age × diet ($p < 0.01$), teeth × diet ($p < 0.001$), and teeth × age × diet ($p < 0.001$).

Table 1 shows the parameters of the six equations for the calculation of probabilities of observation of a particular THS score, given a particular combination of factors. These equations have the generic formula:

$$P\left(THS_n\right) = \frac{\exp(\beta_{0n} + \beta_1 + \beta_2 + \beta_{1,2} + \beta_{1,3} + \beta_{2,3} + \beta_{1,2,3})}{1 + \exp(\beta_{0n} + \beta_1 + \beta_2 + \beta_{1,2} + \beta_{1,3} + \beta_{2,3} + \beta_{1,2,3})} \tag{1}$$

where $P(THS_{\mathrm{n}})$ is the probability of a particular THS (n scoring 1 to 6), $\beta_{0\mathrm{n}}$ is the threshold parameter for each one of the six equations (n scoring 1 to 6), β_1 is the parameter for type of teeth, β_2 is the parameter for diet, $\beta_{1,2}$ is the parameter for teeth × diet, $\beta_{1,3}$ is the parameter for teeth × age, $\beta_{2,3}$ is the parameter for diet × age, and $\beta_{1,2,3}$ is the parameter for teeth × diet × age.

Table 1. Parameters estimation for the equations used to calculate the probability of each of the combinations of teeth type, diet, and age, to score each of the THS values.

Variable		Parameter Estimation (β)	SE
Threshold	THS_6	−3.391	1.408
	THS_5	−2.492	1.334
	THS_4	−1.166	1.287
	THS_3	0.154	1.263
	THS_2	1.680	1.233
	THS_1	2.432	1.280
Teeth	Incisors	1.948	2.184
	Canines	0.767	1.777
	Premolars	−2.993	1.927
	Molars	0	
Diet	Dry	−0.051	1.477
	Wet	−1.826	1.451
	Dry + Wet	1.686	0.618
	Homemade	0	

Table 1. *Cont.*

Variable	Parameter Estimation (β)	SE
Incisors × Young	−0.121	1.7749
Incisors × Adult	0.699	1.1050
Canines × Young	0.150	1.3088
Canines × Adult	0.091	0.9347
Premolars × Young	2.402	1.3880
Premolars × Adult	2.263	0.9023
Molars × Young	−0.477	1.5227
Molars × Adult	−2.035	1.2142
Young × Dry	2.138	2.080
Young × Wet	1.298	2.176
Young × Dry + Wet	−3.038	1.063
Adult × Dry	3.010	1.511
Adult × Wet	2.107	1.463
Incisors × Dry	1.220	2.345
Incisors × Wet	2.914	2.402
Incisors × Dry + Wet	−2.241	1.308
Canines × Dry	−0.175	1.833
Canines × Wet	0.434	2.071
Canines × Dry + Wet	−1.898	0.938
Premolars × Dry	2.553	2.061
Premolars × Wet	2.125	2.045
Premolars × Dry + Wet	−0.864	0.625
Incisors × Young × Dry	−1.440	2.945
Incisors × Young × Wet	−1.593	2.963
Incisors × Young × Dry + Wet	5.461	2.191
Incisors × Adult × Dry	−2.942	2.489
Incisors × Adult × Wet	−5.459	2.543
Canines × Young × Dry	0.729	2.089
Canines × Young × Wet	−0.255	2.481
Canines × Young × Dry + Wet	0.505	1.227
Canines × Adult × Dry	−0.755	1.916
Canines × Adult × Wet	−2.343	2.136
Premolars × Young × Dry	−1.768	2.581
Premolars × Young × Wet	−1.226	2.628
Premolars × Young × Dry + Wet	0.978	1.387
Premolars ×Adult × Dry	−3.023	2.075
Premolars × Adult × Wet	−3.016	2.060

Table 2 gives the different odds ratio readings as probabilities of scoring each of the THS for the different combinations of type of teeth, diet, and age. These are the probability values calculated after application of the generic formula previously introduced.

The odds ratio increases with the value of the parameter, therefore, as can be observed, and as expected, the odds ratios for a lower score are higher than those of a higher score (threshold for THS_1 = 2.432 and for THS_6 = −3.391, with the others ordered in between). This principle could be applied to the variables in the model, provided that no interaction was observed; once this was not the case, the odds ratio for the different variables needs to be contextualised within the interaction.

Table 2. Probabilities of THS score in dependency on the different combinations between the levels of the factors analysed (teeth, age, diet). Probabilities are in ascending order to aid reading.

Variables			**Probabilities for the Different THS Scores**					
Teeth	**Age**	**Diet**	**6**	**5**	**4**	**3**	**2**	**1**
incisors	adult	dry	0.001	0.002	0.006	0.023	0.099	0.190
incisors	young	dry	0.001	0.002	0.008	0.028	0.118	0.221
incisors	young	dry + wet	0.001	0.002	0.008	0.028	0.118	0.221
canines	young	dry	0.001	0.002	0.009	0.032	0.133	0.245
incisors	old	dry	0.001	0.004	0.014	0.049	0.192	0.335
incisors	old	wet	0.002	0.004	0.015	0.053	0.205	0.353
canines	adult	dry	0.002	0.005	0.017	0.061	0.230	0.388
incisors	adult	homemade	0.002	0.006	0.022	0.076	0.275	0.446
incisors	young	wet	0.002	0.006	0.022	0.078	0.281	0.453
premolars	young	dry	0.003	0.008	0.031	0.106	0.354	0.538
incisors	adult	dry + wet	0.004	0.010	0.037	0.126	0.398	0.584
incisors	young	homemade	0.005	0.013	0.048	0.158	0.463	0.647
premolars	adult	dry	0.006	0.014	0.051	0.167	0.480	0.662
molars	old	dry + wet	0.006	0.015	0.055	0.178	0.498	0.678
molars	young	dry	0.007	0.016	0.059	0.189	0.517	0.695
incisors	old	dry + wet	0.008	0.020	0.072	0.225	0.571	0.739
molars	adult	dry	0.013	0.032	0.110	0.316	0.680	0.819
canines	young	homemade	0.013	0.032	0.111	0.318	0.682	0.820
canines	adult	homemade	0.014	0.034	0.117	0.331	0.695	0.828
canines	adult	dry + wet	0.017	0.042	0.140	0.379	0.738	0.856
canines	young	wet	0.019	0.045	0.150	0.398	0.753	0.866
canines	old	dry + wet	0.019	0.045	0.152	0.401	0.755	0.867
canines	old	dry	0.019	0.046	0.154	0.404	0.757	0.869
incisors	adult	wet	0.022	0.053	0.175	0.443	0.785	0.886
premolars	adult	dry + wet	0.030	0.070	0.221	0.515	0.830	0.912
molars	old	dry	0.034	0.080	0.247	0.551	0.850	0.923
premolars	young	wet	0.040	0.094	0.280	0.592	0.870	0.934
molars	adult	dry + wet	0.046	0.105	0.306	0.623	0.884	0.942
molars	young	homemade	0.051	0.118	0.334	0.653	0.896	0.948
premolars	old	dry	0.052	0.119	0.337	0.656	0.898	0.949
premolars	young	homemade	0.057	0.130	0.360	0.678	0.906	0.954
canines	old	wet	0.059	0.134	0.368	0.685	0.909	0.955
premolars	adult	homemade	0.065	0.147	0.393	0.708	0.918	0.959
canines	adult	wet	0.068	0.152	0.402	0.716	0.921	0.961
molars	young	wet	0.084	0.184	0.460	0.761	0.936	0.969
premolars	adult	wet	0.114	0.24	0.543	0.817	0.953	0.978
molars	adult	wet	0.163	0.323	0.643	0.871	0.969	0.985
molars	old	wet	0.173	0.339	0.659	0.879	0.971	0.986
canines	young	dry + wet	0.173	0.340	0.660	0.879	0.971	0.986
premolars	young	dry + wet	0.173	0.340	0.660	0.879	0.971	0.986
molars	young	dry + wet	0.173	0.340	0.660	0.879	0.971	0.986
molars	adult	homemade	0.205	0.388	0.705	0.899	0.976	0.989
premolars	old	dry + wet	0.228	0.420	0.732	0.911	0.979	0.990
premolars	old	wet	0.332	0.550	0.822	0.945	0.988	0.994

THS: teeth health status; Note: Probabilities including the combinations of "old age" and "homemade diet" were not computed as this combination was inexistent in the sample.

3.2. Discussion

In a very generic way, it can be observed that incisors of young or adult cats with a dry diet have lower probabilities of scoring a high TSH value, in comparison to the premolars and molars (cheek teeth) of older cats having a diet with a wet component. Incisors score even lower in older cats, independently of type of food. Cheek teeth score higher, even in young cats, but predominantly where wet commercial food or homemade food (which is also wet/soft) was fed. Age is shown to be the least predominant factor of the three considered. Diet and type of teeth were found to be the predominant and determinant factors responsible for the variability in scores, and, therefore, cat oral health status.

Cheek teeth are larger and hide in the buccal cavity and are difficult to be cleaned by the tongue, which explains the accumulation of food debris, which builds up the bacterial pool and encouraging the development of both plaque and gingivitis at higher frequencies. Cheek teeth also play a predominant role in mastication, and, as the incidence of diastemata in these teeth is more common than in the incisors, the creation of food pockets may prevail. One last aspect differentiates cheek teeth and incisors in cats: the role played by incisors in corporal hygiene, which contributes to a higher degree of abrasion and, therefore, the prevention of plaque formation.

Several authors have previously identified wet or soft food as being responsible for a lower health status of teeth [4,10,13,14] when compared to dry food. The explanation for this fact stays with the abrasive properties of dry food, capable of removing the teeth plaque. Clarke and Cameron [6] have studied the impact of a commercial diet (mixed, wet, and dry) in domestic cats, by comparing them with their feral cat free hunter cousins, but found no significant differences between them in calculus formation and degree of PD. These authors concluded that commercial food cannot be solely responsible for the development of PD in cats. However, Gorrel *et al.* [15] demonstrated the beneficial effect of the addition of a "dental hygiene chew" feed in terms of prevention of plaque and calculus accumulation on tooth surfaces, even when cats were fed dry food.

Dry food can eventually be responsible for an increased production of saliva. It is well known that saliva contains immunoglobulins produced in reaction to the antigens found in the mouth [16], and, therefore, dry food eventually will also be responsible for a better use of the immune system in the prevention of oral health issues.

Good oral hygiene has proven to prevent the development of PD in cats, and several authors refer to that in review articles (e.g., [3]) and research articles after trials (e.g., [1]). It has been identified that it is difficult to habituate a cat to dental hygiene methods and it has been suggested that an early habituation, from the kitten stage, is fundamental. The results of the present study show that, even at an early age, cats are susceptible to poor health status in their cheek teeth, especially if eating wet food. It is suggested that good hygiene of the feeding bowl should also be considered to avoid bacterial build up, especially where cats are fed wet food, as bacteria is the main trigger of plaque development, leading to PD.

As a limitation in this study, we must refer that several other factors may eventually play an important role in cats' oral hygiene. For example, Kornya *et al.* [17] have recently established a relationship between the deterioration of cats' oral health and the positive testing for retroviruses (feline leukaemia virus and feline immunodeficiency virus), and cats in this study were not tested for retroviruses.

In addition, homemade diet nutritional details may vary substantially (nutritional deficiencies, feeding of bones, *etc.*), which may impact on results.

Water intake is another factor with a possible impact on cats' oral health, but the question is not addressed by this study. Cats are animals with a desert origin and the moisture content of food is an important part of water intake. While the moisture content of wet food is over 75%, dry food contains around 6% [18]. Cats fed wet food voluntarily drink small quantities of water while cats fed dry food have a higher voluntary intake [19], which, however, does not compensate for the reduced moisture content [20].

4. Conclusions

Cheek teeth (molars and premolars) are more susceptible to poor oral health than other teeth, independent of the age of the cat. It is important to prevent oral health deterioration from an early age with special attention paid to the cheek teeth. The diet of a cat needs to be considered holistically, paying particular attention to its nutritional value, but the texture of the food is shown to play an important role in oral health, with wet canned food providing the least benefit to oral health.

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Conflicts of Interest

The authors declare no conflict of interest.

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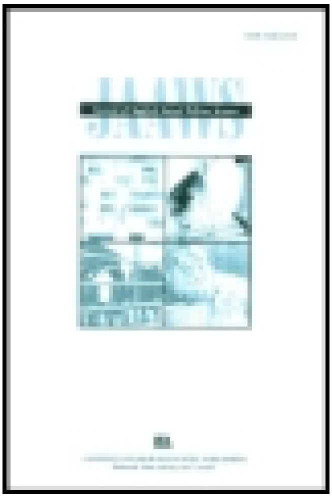

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A Cross-Sectional Epidemiological Study of Prevalence and Severity of Bit-Induced Oral Trauma in Polo Ponies and Race Horses

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A Cross-Sectional Epidemiological Study of Prevalence and Severity of Bit-Induced Oral Trauma in Polo Ponies and Race Horses

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Bit and bridle accessories improperly fitted in ridden horses can cause oral trauma such as bone spurs, commissure ulceration, and tongue lacerations. This study was used to identify, grade, and compare the types of oral traumas commonly found within polo ponies and race horses. Injuries were assessed visually and by palpation on the tongue, lips' commissures, and interdental space. A total of 50 polo ponies and 50 race horses were sampled in the South of England. A Poisson model was successfully fitted to the data ($p < .001$), and the variables of discipline ($p < .001$), injury type ($p < .001$), and age ($p < .001$) were significant. Race horses with snaffle bits were predisposed to significantly higher severities and prevalence of oral trauma than were polo ponies in gag bits. Only polo ponies were observed with tongue trauma. Race horses had higher severities of injuries in the commissures and bone spurs. Positive correlations were found between age and/or time in sport and induced biting injuries. Polo ponies had been playing longer before the occurrence of injuries.

Keywords: *bit and bridle, oral trauma, polo ponies, race horses*

Bits that are ill-fitting, aggressive, or used incorrectly can damage the interdental space and surrounding structures in horses (Clayton & Lee, 1984; Tell, Egenvall, Lundstrom, & Wattle, 2008). Oral trauma such as bone spurs, ulceration, and tongue lacerations due to bit and bridle accessories are common among ridden horses (Clayton & Lee, 1984; Tell et al., 2008). Due to the thin bars of the mandible, stamina, and stresses of work, polo ponies and race horses could be predisposed to oral trauma (Johnson, 2002). There is, however, little or no evidence of the extent of oral trauma suffered within these sports. Pain on palpation, facial neuralgia, and avoidance of the bit are common signs of discomfort within the oral cavity (Cook, 1999, 2003; Mills & McBride, 2002).

Bits that are used widely today have to adhere to modern ethics that are standardized by the Animal Welfare Act of 2006. This act inhibits the use of severe mouthpieces (e.g., wired or spiked) that may inflict pain/injury during equitation (Cook, 2002). There is great debate,

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unsubstantiated by scientific evidence, as to the ethical implications of using any bit (Cook, 2002, 2003). Cook (2002) has been reviewing the subject and suggests that most behavioral problems stem from the bit inflicting pressure on the numerous sensory nerves that originate in the oral cavity, thereby causing pain.

Anecdotally, polo ponies are ridden with a gag and race horses with a snaffle, suggesting they are bitted for the sport as opposed to the individual horses' bitting requirements (e.g., oral conformation). However, although rules and regulations of the Federation of International Polo and the British Horse Racing Authority prohibit the use of bitless bridles within competition, it appears there are no regulations for the types of bitting used except the discretion of the veterinary surgeon attending (British Horseracing Authority, 2012; Federation of International Polo, 2010; Hurlingham Polo Association, 2014). Hurlingham Polo Association (2014) states that a pony may not be played if the pony is not under proper control or shows a vice that is a danger to other ponies or players; thus, it may be assumed that the common use of a gag is to gain maximum control over the nonhuman animal and prevent removal from a game. Within racing, no such rule appears to be stated, suggesting unknown reasons regarding the reliance on the snaffle. Cook (2002) suggests that race yards are likely to have 20% of horses suffering from oral trauma at one time, which reduces performance of the overall yard and therefore suggests that the industry's perceived reliance on the snaffle is inappropriate despite its anecdotally mild nature.

There are many different variations of the snaffle including number of links, shape, and bit rings; however, all snaffles have the cheek pieces of the bridle and reins attach to the same or adjacent bit rings (Evans & Barnett, 2007b). The snaffle is a nonleverage bit with a jointed or a solid mouthpiece providing a direct signal, which is equal in its pressure to that directed from the rider's hands to the horse's mouth (1:1 pressure ratio), making the snaffle the mildest bit (Cook, 2002; Evans & Barnett, 2007b). The gag reins are attached lower than the mouthpiece to provide leverage and create up to 3 times additional pressure than the pressure applied through the rein when compared with the snaffle, which if used incorrectly can inflict severe oral trauma (D. Bennett, 2006; G. Bennett, 2001; Evans & Barnett, 2007a).

When in action, the gag's mouthpiece encourages the horse to lift his or her head upward through a lever action relieving pressure at the tongue and bars, sliding the bit upward in the mouth by applying pressure on the lips and poll through the headpiece (G. Bennett, 2001). Lengthening the shanks of the gag increases leverage and severity; however, it also gives the horse increased signaling and faster pressure relief, which may limit oral trauma (G. Bennett, 2001).

Engelke and Gasse's (2003) study of cadavers showed that the tongue would take the full force of the bit by splaying upon rein tension to reduce pressure on the bars. Thus, it is suggested that the horses who lean on the bit may increase lingual compression to relieve bit pressure from the bars, resulting in a potential increase in the prevalence of tongue lacerations (Engelke & Gasse, 2003; Manfredi, Clayton, & Rosenstein, 2005). Acute trauma to the bars resulted in edema and, if severe, bony sequester or exostosis (bone spurs; Johnson & Porter, 2006). Buccal ulceration is also commonly seen in ridden horses due to the sharp enamel points interacting with the bridle. Tell et al. (2008) found 100% of ulcers opposing the 06s of the ridden snaffle-bitted horse when compared with only four ulcers seen in 20 unridden horses.

The aim of this study was to identify, grade, and compare three types of oral traumas commonly found within polo and racing by palpation and visual assessment of the rostral portions of the tongue, commissures of the lips, and interdental space close to the 06s and 07s. Grading systems for these three types of injuries were developed and used to relate to other demographic variables and types of discipline.

MATERIALS AND METHODS

Polo ponies are normally horses (1.5 m median withers height, according to Brooks et al., 2010), but they are commonly called ponies by their riders. We used the names "polo ponies" and "race horses" to allow for an easier differentiation between the two groups. This study was carried out after data were collected in Britain from four polo and four flat race yards at Gloucestershire and the Southeast. From these, 50 polo ponies and 50 race horses were sampled by convenience after agreement with yard owners and trainers at the time of data collection. The trainer for each yard confirmed the bits in which each horse was ridden, and only polo ponies ridden in gag bits and race horses ridden in snaffles were utilized for this study. Polo ponies' ages ranged from 3 to 23 years old, and race horses' ages ranged from 4 to 11 years old.

The mouths of the horses were assessed for injuries as bone spurs, injuries in the commissure, and tongue injuries through palpation of the interdental space and commissure, while tongues were visually assessed. The horses were not sedated during this examination process. Horses assessed were graded individually, on both the left and right sides, using an adaptation of the grading system developed by Cook (2011) for bone spurs and an original grading system developed for this project (see Tables 1 and 2).

Other information collected included ages of the horses, times in sport, genders, and bit types. Horses with histories of pathologies, such as fractured jaws or clinical signs of disease (e.g., swollen glands), were not included in the analysis.

A generalized linear model from the Poisson family with a log-link function was fitted to the data to model grade as the dependent variable. The following were used as independent variables: discipline (polo, race), injury type (commissure, tongue, and bone spurs), side (left, right), gender, age, and time in sport. A backward stepwise procedure for selection of variables was implemented after the chi-square Wald test, with the significance level set to $p < .05$. Interactions between significant factors were also tested. The model was assessed for significance after the likelihood ratio chi-square test. Data were analyzed with the generalized

TABLE 1
Grading System Used to Determine Injuries in Lips' Commissures and Interdental Space Close to 06s and 07s

Grade	*Definition*
0	No signs of mucosal damage
1	Some signs of pinkness, abrasion, decoloration
2	Break just in the surface of the skin
3	Large break in the surface of the skin or blistering
4	Increasing depth of tissue damage that crosses a large width of the cheek and blistering skin structures
5	Scarring tissue, deep damage to tissue structures, bleeding, or signs of recent damage (e.g., blistering, healing tissue structures)

TABLE 2
Grading System Used to Determine Injuries in the Tongue

Grade	*Definition*
0	No signs of mucosal damage
1	Some signs of pinkness, abrasion, and discoloration
2	Break in the surface of the skin
3	Large break in the surface of the skin
4	Increasing depth of tissue damage that crosses a large width of the tongue
5	Missing portions of the tongue or complete laceration and/or scarring

linear models routine of the IBM Statistical Package for the Social Sciences Version 21 software. Ethical approval was gained from the Hartpury College Ethics Committee in advance.

RESULTS

The distribution of the scores for the different traumas and disciplines is represented in Table 3. The Poisson model was successfully fitted to the data (likelihood chi-square = 162, 6 *df*, $p < .001$). The factors of discipline (Wald chi-square = 51, 1 *df*, $p < .001$) and injury type (Wald chi-square = 18, 2 *df*, $p < .001$) were found to be significant, together with the interaction between these two factors (Wald chi-square = 12, 1 *df*, $p < .01$). The covariate of age was also found to be significant (Wald chi-square = 35, 1 *df*, $p < .001$), together with an intercept (Wald chi-square = 91, 1 *df*, $p < .001$). Full parameters of the model are presented in Table 4, with Wald 95% confidence intervals.

Figure 1 illustrates the differences found in injury ratings, disciplines, and variability with age and was drawn using the generic equation $exp\ (\beta_{0+}\beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1 X_2 + \beta_4 X_4)$, where β_0 is the intercept; β_1 is the parameter associated with the factor discipline; β_2 is the parameter associated with the factor injury type; $\beta_{1,2}$ is the parameter associated with the interaction between the factors of discipline and injury type; β_3 is the parameter associated with the covariate

TABLE 3
Number of Observations Per Discipline, Type of Trauma, and Trauma Score

		Score					
Discipline	*Trauma*	*0*	*1*	*2*	*3*	*4*	*5*
Polo ponies	Tongue laceration	94	6	0	0	0	0
	Commissure ulceration	85	7	5	3	0	0
	Bone spurs	72	15	9	3	1	0
Race horses	Tongue laceration	100	0	0	0	0	0
	Commissure ulceration	47	24	16	6	2	5
	Bone spurs	70	7	10	10	1	2

Note. Fifty horses were sampled per discipline and were scored on the left and right sides of the mouth, and therefore, totals add up to 100.

TABLE 4
Parameters of Fitted Poisson Regression of Injury Grade for Dependency of Type of Injury, Discipline, and Age

Parameter	*β*	*SE*	*95% Wald CI*		*p Value*
Intercept	−30.962	.557	−32.054	−29.870	<.001
Discipline					<.001
Polo	26.967	.311	26.358	27.575	
Race	0				
Injury					<.001
Bone spurs	29.793	.545	28.725	30.860	
Commissure	30.249	.485	29.300	31.199	
Tongue	0				
Age	0.112	.023	0.067	0.157	<.001
Discipline × Condition					<.01
Polo × Bone Spurs	−27.744	.385	−28.500	−26.989	
Polo × Commissure	−28.720	.376	−28.344	−29.096	
Polo × Tongue	0				
Race × Bone Spurs	0				
Race × Commissure	0				
Race × Tongue	0				

Note. The model has an Akaike's information criterion of 727.

age; X_1, X_2, and $X_{1,2}$ are the dummy variables associated respectively with discipline, injury type, and the interaction between discipline and injury type; and X_3 is the age of the horse or pony.

The severity increased with age, and the conditions were more prevalent in race horses compared with polo ponies. Commissure injuries were more prevalent in race horses and had higher severities than bone spurs in horses of both disciplines. Tongue injuries showed low prevalence with some observed in polo ponies only ($n = 4$ horses).

DISCUSSION

Both polo ponies and race horses suffered oral trauma; however, there are significant differences between the discipline and the injuries sustained. Race horses using snaffle bits sustained overall higher occurrences and significantly different levels of oral trauma severities when compared with polo ponies using gag bits ($p < .001$). The race horses used the snaffle bit while polo ponies used the gag bit, and therefore, discipline and bit type confound each other as variables in terms of buccal injuries. Warren-Smith, Curtis, Greetham, and McGreevy (2007) found that higher rein tensions occurred during the halt (1.62 kg) and turning phases of riding (mean of 0.75 kg); thus, it might have been expected that polo ponies had higher injuries due to the nature of the sport involving turning and stopping abruptly.

The standard loose ring jointed snaffle, which is commonly used in racing, may place pressure directly upon the bars, the tongue, and the commissures (Manfredi et al., 2005). The gag anecdotally relieves pressure from the interdental space upon rein contact and redistributes the pressure onto the commissures and the tongue (Evans & Barnett, 2007a). Manfredi et al. (2005) found that the topography of bits changes within the oral cavity upon rein tension and varies between bit types (e.g., linked or straight mouthpiece). Thus, pressure exertion sites on the oral

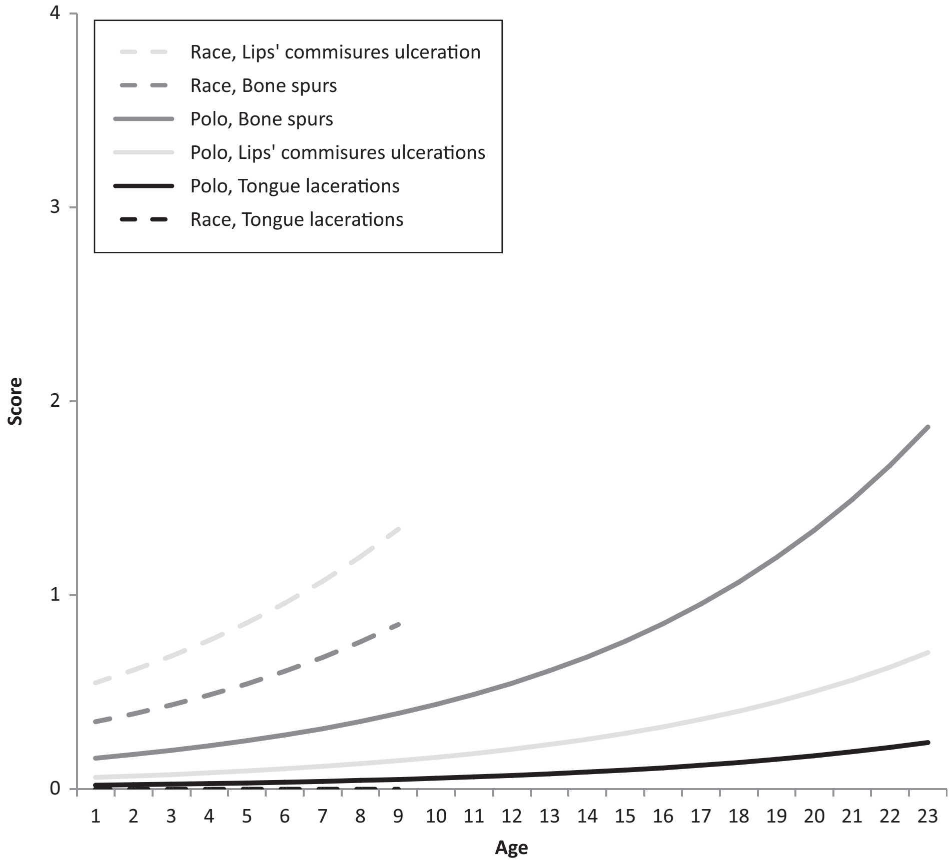

FIGURE 1 Graph of the fitted model illustrating the differences found in injuries within horse disciplines and their variability with age. The model is valid for ages ranging from 3 to 23 years old for polo ponies and 4 to 11 years old for race horses.

structures may differ between mouthpieces and may predispose certain areas to injury depending on bit type (Manfredi et al., 2005). This study supports these findings through the identification of oral trauma at differing sites between bits. The snaffle bit implemented trauma upon the commissures and the bars, whereas the gag produced oral trauma in these areas.

Different trainers and methods of training (e.g., light hands) may cause horses to respond to varying pressures before performing a desired response, and therefore, we have identified a potential source of bias in the study. Intuitively, we can say that horses who have been trained to respond to stronger aids may encounter more oral injuries than those given lighter aids, due to repeated increased pressures of the bit on the oral structures. Individual horses also have varying pressure thresholds that must be reached before performing a desired response (Clayton, Singleton, Lanovaz, & Cloud, 2003).

The polo pony anecdotally is broken in relatively slowly to enable horses to learn rider aids and respond to them quickly in order to change direction and follow the ball at speed. Therefore, despite the gag's anecdotal severity, horses are taught to respond to the signal that the leverage bit provides before too much pressure is enforced, thereby limiting oral trauma (D. Bennett, 2006). However, race horses are anecdotally broken in quickly and at relatively young ages with little schooling, suggesting that the horse's responses to rider aids are not as well developed as those of the polo pony. Therefore, race horses may be less responsive to the bit, thereby needing larger rein pressures and causing the oral trauma that was seen.

Race horses had a significantly higher prevalence of commissure damage ($p < .001$) and higher severity grading. These results may have been due to dental malocclusions such as hooks on the 06s, which have been suggested to increase impingement of the commissure between the bit and the 06 premolar. Brigham and Duncanson (2000) found that 26% of the horses studied had focal overgrowths on the 06s, supporting Allen's (2004) findings. Pence (2002) suggested that bit seating can limit the occurrence of soft-tissue impingements against the 06s; however, there is limited research into its advantages. The fact that bit seats are anecdotally performed to an aggressive standard in the polo pony may suggest that this provision limited oral trauma (Pascoe, 2010).

Prophylactic dentistry of the race horse should be reviewed to include bit seating to the same degree as the polo pony, thus reducing the prevalence of ulcerations (Pascoe, 2010; Scoggins, 2001). However, as of yet, there is no scientific evidence to fully support the application of bit seating to the rostral aspect of the 06s to enhance performance and reduce the risk for lacerations to the oral mucosa surrounding the area. The procedure of bit seating or rostral rounding also needs to be completed with extreme caution, as it has been shown that the pulp chambers located in the rostrodorsal aspect of the 06s can lie very closely to the occlusal surface (Williams, McGuarian, & Johnson, 2011). Tell et al. (2008) found 100% of ulcers opposing the 06s of the ridden snaffle-bitted horse when compared with four ulcers in 20 horses who had not been not ridden. However, the study did not state in which discipline the horses ran.

From the three places evaluated for trauma, tongues were the least affected; they were not affected at all in race horses and were slightly affected in polo ponies. D. Bennett (2006) provided radiographs of the gag in action, suggesting that under rein tension, the gag places pressure onto the tongue. Manfredi et al. (2005) also found that the snaffle bit places pressure upon the tongue under rein tension, causing the horse to retract or bulge the tongue over the bit. This provides relief from bit pressure on the hard palate or provides the tongue relief from bit pressure from itself and distributes some of the pressure onto the interdental space (Engelke & Gasse, 2003; Manfredi et al., 2005). Unlike the snaffle, there is little research into the gag's action on oral structures under rein tension, and so without radiographic evidence, it can only be assumed that the gag does not permit the retraction of the tongue as easily as does the snaffle.

Cook (2011) found 62% of domestic mandibles had bone spurs, and this finding corresponded with findings from Van Lancker, Van der Broeck, and Simoens's (2007) study (48%). Race horses were found to be more prone to bone spurs compared with polo ponies. D. Bennett (2006) suggested the gag places pressure upon the commissures to elevate the head, thereby enabling the horse to shift his or her weight onto his or her hindquarters and stop sharply. Van Lancker et al. (2007) suggested that the bit may place a caudal traction force upon the buccinator muscle's point of insertion at the interdental space during rein tension, resulting in periosteal remodeling that may be the cause of bone spur formation within polo ponies, despite its limited pressure exerted onto the bars during rein tension. It is possible that race horses also suffer bone spurs in this way; however, due to the topography of the snaffle, it is more likely that it exerts direct pressure onto the bars under rein tension, resulting in bone spur formation (Manfredi et al., 2005).

Age was found to correlate positively with all the buccal injuries. Comparatively and generally, race horses encounter oral trauma at a younger age. Thus, the results suggest that polo ponies had better oral mucosal health when fitted with a gag bit and received fewer injuries than the race horse in the anecdotally milder bit. It may be suggested that this relationship was encountered due to the much younger starting age of race horses.

The positive correlation between increased age/time in sport and developing oral trauma in polo horses may be due to the habituation to bit pressure (Warren-Smith et al., 2007). McGreevy and Rodgers (2005) suggested rein contact should be light; however, over time, the horse may habituate to the pressure placed upon the oral structures from the bit and cause the rider to use more force down the rein to achieve the same desired response, thus increasing pressure upon oral structures during rein contact and resulting in increased oral trauma over time. As race horse yards interchanged complete bridles including the bits between horses, the faster rates of oral trauma may be directly related to the bits' poor fit such as pinching the commissures (if the bit was too small) or sliding within the cavity (if the bit was too big), thereby causing the bit to be pulled through the mouth and creating trauma (G. Bennett, 2001).

CONCLUSION

As flat race horses may be trained and raced at younger ages than polo or National Hunt horses, the ages of the horses, the associated changes from deciduous to adult teeth, and the effect they have on their oral mucosal health should be considered within future research on this subject area (Allen, 2004).

Race horses with a snaffle bit were found to be predisposed to significantly higher severities of oral trauma and a higher prevalence of oral injuries compared with polo ponies in a gag bit. Injuries sustained by the bit varied in topography, depending upon the bit's application of pressure when under rein tension. The gag bit was the only bit type where the potential for associated tongue trauma was recorded.

Snaffle bits in race horses inflicted a higher degree of injuries in the commissure and also caused a higher degree of bone spurs than in polo ponies who had gag bits. Positive correlations were also found between age and/or time in sport and induced bitting injuries. Generally, polo ponies had been playing longer before injuries occurred, whereas the race horses incurred injuries in a much shorter time frame.

As recently reviewed by Campbell (2013), the discussion of "when use becomes abuse in equestrian sport" promotes debate, with some arguing that animals should not be used in sports at all and others suggesting that this is morally accepted, provided the animals are "humanely" treated. The boundaries between use and abuse may not be well established yet, but the promotion of animal welfare shall always be an aim of a developed society. With this study, we hoped to have made a contribution to the debate and to the identification of competition horse welfare issues that need to be addressed.

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Original Research

Evaluation of Horse Fitness for Exercise: The Use of a Logit-Log Function to Model Horse Postexercise Heart Rate Recovery

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ABSTRACT

The aim of this study was to test the adjustment of the logistic-log model with post-exercise heart rate (HR) recovery data in horses and introduce the logit-log model as a parsimonious model, with the half recovery time as a measure of horse fitness. Post-exercise HR (bpm) measurements at different timings were obtained from 32 polo ponies in two different exercise regimes: n = 16 “full chukker” and n = 16 in “half chukker.” Measurements were taken at rest, immediately after exercise and then 2, 4, 6, 10, and 20 minutes after exercise. The HR variable was transformed into a logit (HR) variable, and time was transformed into log (time). Means of logit (HR) at the different log (times) were obtained, creating two time series of transformed variables that were then adjusted by simple linear regression. The degree of adjustment of the model is high with values of $r^2 = 0.989$ for the full chukker and $r^2 = 0.998$ for the half chukker. Full chukker ponies have a half recovery time of 5.7 minutes and half chukker ponies of 11.2 minutes, with the former showing to be fitter than the latter, which is why they are chosen to play for longer periods. The logit-log model is parsimonious, and the half recovery time can be easily determined. The half recovery time has potential to be used as a measure of fitness degree, allowing comparison between horses.

1. Introduction

Postexercise heart rate (HR) recovery has been used as an indicator of fitness because of the positive correlation between recovery and overall physical performance of individuals [1,2]. Several studies have demonstrated the relationship between quicker recovery and better performance [3]. The mono and biexponential models have been of choice in HR recovery modeling [4], with the biexponential often proving to be the best fit [5]. The biexponential model results in a best fit owing to the fast and slow components of HR recovery observed. After exercise, at peak HR, the immediate recovery decays at a fast pace, whereas later, this pace slows down until the resting HR is reached [5]. The exponential model was proposed by Malthus to model demographic growth [6], and the biexponential model is an extension of this, breaking it in two components. These models can either model growth or decay, by making its growth rate parameter positive or negative. The two components of the biexponential model are supported by the theory of coordinated interaction of parasympathetic reactivation and sympathetic withdrawal during exercise recovery as explained by Bitshnau et al [2], therefore allowing a biological interpretation of the parameters of the model. But as these same authors state, several other authors have studied the subject inconclusively [7], and this interaction between the two parts of the autonomic nervous system is still unclear in both man and horse.

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Verhulst [8] extended the Malthusian growth model with a ceiling parameter originating the logistic model. The logit is the inverse of the logistic model, and was proposed to model bioassay in 1944 by Berkson [9]; since then, its application has been growing in biological sciences but also in econometrics and social sciences [9]. The logit-log function is a model of choice to model decay (e.g., in radioimmunoassay) and was proposed to model the post-exercise HR recovery and oxygen uptake in humans by Stupnicki [10].

In this study, horse HR recovery is fitted by the logit-log model to investigate the adjustment of data and test the application of the model as a measuring tool of horse fitness for exercise. The model is tested in polo horses, which are full-size horses, but traditionally called ponies.

Polo is considered a high demand sport with moderate-to-high stress on the cardiovascular system of the pony [11], with polo ponies working at high HR for a high proportion of the time [12]. Heart rate of horses can reach approximately 200 bpm at peak [2] and should be approximately 35 [12] when resting. Physically fit individuals will have a lower HR at rest and a faster recovery [1]. A polo match consists of four parts, known as chukkers. Each chukker lasts for 7.5 minutes. Each rider can either play one ("full chukker") or two ("half chukker") ponies in each chukker, playing each pony for 3.5 minutes. Any pony can only reenter the game after the resting period of a full chukker (or two half chukkers).

2. Materials and Methods

2.1. Subjects and Data Collection

Data were collected in N = 32 polo ponies of polo clubs across the south west of England, using a Polar WIND HR transmitter and monitor, to measure HR. Each polo pony's resting HR was measured before play and then immediately after playing to determine peak HR measurement, then 2, 4, 6, 10, and 20 minutes after play. All 32 ponies are Thoroughbred mares, the majority Argentinean, with six mixed breed. Ages varied between 3 and 17 years, height from 15 to 16.2 hands, and all the ponies were deemed fit to play. From these N = 32 ponies, n = 16 are full chukkers and the other n = 16 are half chukkers.

2.2. Processing of Data

The variables "time" and "HR" were transformed within MS Excel using log (time) and logit (HR), respectively. Logit (HR) was obtained using the formula:

$$logit(HR) = log\left(\frac{HRt}{HR_p - HR_t}\right) \tag{1}$$

where HR_t is the HR at time t and HR_p is the peak HR obtained immediately after exercise. Both these values are net values, obtained by subtracting HR_0 (HR at rest) measured before the start of the exercise. All the transformations were made using decimal logarithms. After the transformations of the variables, the means logit (HR) for each log (time) were calculated to create time series for both the half and full chukkers, as shown in Table 1. The standard deviations and 95% confidence interval for the means were also computed.

2.3. Statistical Analysis

Simple linear regressions were performed between log (time), as independent variable, and means logit (HR), as dependent variable, for both half chukker and full chukker. The fitness of the models was evaluated through the coefficient of determination (r^2). The normality of the distribution of the residuals was assessed through a normal probability–probability plot.

Significant differences between half chukkers and full chukkers, for both HR at rest and peak HR, were tested with independent samples t tests.

All the analysis was performed using the statistical package IBM SPSS Statistics 20.

3. Results

No significant differences ($P < .05$) were found between half chukkers and full chukkers for both HR_0 and HR_p.

The linear regressions adjust very well, with a high coefficient of determination in both cases: $r^2 = 0.989$ and $r^2 = 0.998$ for full and half chukkers, respectively. In both regressions, both the intercepts and the slopes are highly significant ($P < .001$), and the analysis of the normal probability–probability plots of the residuals did not suggest any deviance from normality. The adjustment by the

Table 1
Series with mean HR measured at the indicated timings, respective SD, and 95% CI for ponies playing half and full chukkers

Time		Half Chukkers				Full Chukkers			
Minutes	log	Mean HR	Mean Logit	SD	95% CI	Mean HR	Mean Logit	SD	95% CI
2	0.301	97	1.12	0.26	±0.13	86	0.81	0.36	±0.18
4	0.602	91	0.70	0.25	±0.12	76	0.30	0.25	±0.12
6	0.778	84	0.39	0.18	±0.09	67	−0.02	0.24	±0.12
10	1.000	73	0.05	0.10	±0.05	56	−0.38	0.29	±0.14
20	1.301	60	−0.36	0.22	±0.11	44	−1.12	0.37	±0.18
Rest		40				39			
Maximum		102				95			

CI, confidence interval; HR, heart rate; SD, standard deviation.
The table also shows the series with the transformed variables log (time) and logit (HR).

Table 2
Parameters of the fitted models for both half and full chukkers

Model	*P* Value	r^2	Parameter	*P* Value	Coefficient	SE	95% CI
Full chukker	<.001	0.989	Intercept	<.001	1.432	0.086	1.159–1.706
			Slope	<.001	−1.902	0.099	−2.218 to −1.586
Half chukker	<.001	0.998	Intercept	<.001	1.573	0.035	1.463–1.683
			Slope	<.001	−1.499	0.040	−1.626 to −1.371

CI, confidence interval; SE, standard error.
The coefficients of determination for the fitted biexponential model using the same data series.
Note: With the biexponetial model, $r^2 = 0.805$ for full chukker and 0.974 for half chukker.

biexponential model was also fitted with high coefficient of determination but showing slightly lower values, in comparison with the logit-log model (0.974 and 0.805 for full and half chukkers, respectively). The full results of the regression analysis can be found on Table 2 and visualized in the plot of Fig. 1.

4. Discussion

The logistic distribution, using decimal logarithms, is written as $F(z) = 1/(1 + 10^{-z})$, being z any number. This distribution can easily produce a linear logistic model if z is made equal to a linear equation. Using the straight line equation $z = \alpha + \beta X$, we arrive at

$$F(\alpha + \beta X) = \frac{1}{1 + 10^{-(\alpha+\beta X)}} \tag{2}$$

The inverse linearizing transformation of the logistic model Equation (2) is the linearized logit model and is written:

$$F^{-1}(\alpha + \beta X) = \frac{P}{1 - P} = 10^{(\alpha+\beta X)} \tag{3}$$

where P is a proportion. Taking the logarithms in both sides of the Equation (3) we arrive at

$$log\left(\frac{P}{1 - P}\right) = \alpha + \beta X \tag{4}$$

The logit model is a symmetric model without upper or lower boundaries, and this is why it is seen as a very good

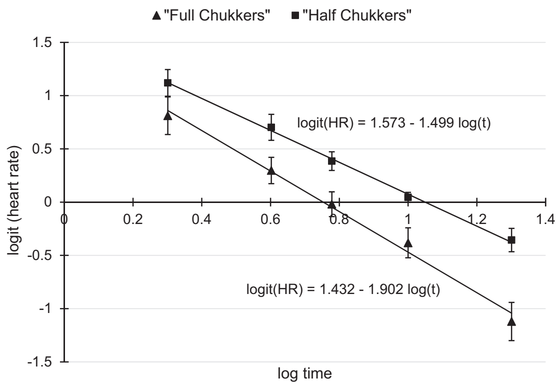

Fig. 1. Plot of the series with mean logit (HR) measured at the indicated log (time), respective SD and 95% CI for ponies playing half and full chukkers. The regression lines and equations from the fitted models were also added. CI, confidence interval; HR, heart rate; SD, standard deviation.

candidate for dependent variable of a linear model of independent variables. The logit model is not a linear relationship, and therefore, the parameter β (the slope of the relationship) is not constant. With the logarithmization of the dependent variable, this relationship is made linear, and therefore, the parameter β is seen as a slope of a straight-line equation, being α the intercept.

In this study, the logit function is built as logit (HR) = log ($HR_t/HR_p - HR_t$). It must be noted that these are net values, and therefore, the value for HR_0 at rest is subtracted from each of the measurements HR_p and HR_t. Finally, the independent variable "time" is logarithmized to arrive at the model used in this study:

$$logit(HR) = log\left(\frac{HR_t}{HR_p - HR_t}\right) = \alpha + \beta(log(t)) \tag{5}$$

It is possible now to plot logit (HR) against log (time) and, as can be observed in Fig. 1, a linear relationship is evident, allowing the adjustment of a linear regression where the intercept α is HR_p and β the slope constant or the decay rate.

The log half-life of the decay can easily be identified in the graph by the point in time where logit (HR) = 0. The log half-life of the decay is the point in time where half of HR_p is achieved, therefore, from Equation (5) and replacing HR_t by $0.5HR_p$:

$$log\left(\frac{0.5HR_p}{HR_p - 0.5HR_p}\right) = log1 = 0 \tag{6}$$

from which we conclude that the log half-life of the decay is achieved when logit (HR) = 0. So, by making

$$\alpha + \beta\left(log\left(\frac{t}{2}\right)\right) = 0,\ we\ arrive\ at\ log\left(\frac{t}{2}\right) = \frac{-\alpha}{\beta} \Leftrightarrow \frac{t}{2} = 10^{-\alpha/\beta} \tag{7}$$

and therefore, using the regression parameters, we can now compute the log half-lives or half recovery times, for the full chukker ponies as 1.432/1.902 = 0.753 and for the half chukker ponies as 1.573/1.499 = 1.049, which is in agreement with the observations taken from the plot in Fig. 1. From here, and by taking the log out, we can compute the real recovery times as $10^{1.499} \approx 11.2$ minutes for the former and $10^{0.753} \approx 5.7$ minutes for the latter.

The model is proved to fit the data very well, with very high coefficients of determination, also enabling the half recovery time to be easily read. The half recovery time is a standardized measurement with a high potential to be used as the comparison unit for fitness degree between horses.

Obviously, as previously discussed by several different authors [13], different factors may interfere with HR increase and recovery during exercise, making it difficult to compare data from different horses without previous data standardizing procedures. However, if the measurements are made in similar exercising situations, they have the same contextualization and therefore are comparable. It also should be noticed, that if the data are fitted well by linear regression, it can be modeled by analysis of variance and covariance. This facilitates the study of significant differences between intercepts and slopes (HR_p and recovery rates), and therefore leading to the calculation of coefficients for factors responsible for differences (e.g., breed, age, gender, and so forth), that can be used in the standardization of data to allow comparability between horses.

It must be noted that the aim of this study is the study of the adjustment of the logit-log model to HR recovery, and therefore, the following should be considered for illustrative purposes only. Considering the data used to test the model in this study as example, we can observe that ponies playing a full chukker have a faster recovery time, and shorter half recovery time, than those playing half chukker. There must be a reason for the use of some ponies in a half and others in a full chukker, and with the analysis performed, it becomes evident that the fitness of the animal may be the reason behind the choice. Ponies playing a full chukker may have a higher degree of fitness compared with those playing a half chukker.

The exponential model is parameterized as $HR(t) = HR_p \exp(rt)$ where r is the decay rate. The biexponential model extends the exponential model breaking it in two components:

$$HR(t) = HR_{p1}exp(r_1 t) + HR_{p2}exp(r_2 t) \tag{8}$$

The adjustment to data of this four-parameter model, through an interactive nonlinear regression procedure, has a much more complex computation than the adjustment of a simple linear regression. The simple linear regression is a more parsimonious model and, considering its high degree of adjustment ($r^2 \approx 0.99$ where achieved), there is no loss of model fitness (r^2 for the biexponential model often reported around the same values [1]). Considering that the biexponential model has been used to allow biological interpretation, which has been shown to be inconclusive [7], there is no loss of information when using a linear regression. The logit-log model makes a transformation of data that enables modeling by simple linear regression.

5. Conclusion

The logit-log model fits very well the HR recovery time in horses, and the half recovery time can be used to measure horse fitness for exercise. The logit-log model is more parsimonious than the biexponential model; it is of easy computation, showing therefore potential to be used in simple portable equipment allowing quick evaluation of horse fitness.

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Publication four

Mata, F. (2014b) Analysis of predisposition factors for limb amputation in dogs with survival analysis in those diagnosed with appendicular cancer. *The Veterinary Nurse*. 5 (7), pp.406-411. DOI: 10.12968/vetn.2014.5.7.406

Analysis of predisposition factors for limb amputation in dogs with survival analysis in those diagnosed with appendicular cancer

Abstract

Aims: This study aims to contribute to the knowledge of epidemiological factors contributing to limb amputation in dogs. A survival analysis for limb amputees from cancerous causes is also performed.

Methods: Data were collected in 2010 from 152 owners of amputee dogs. And a statistical analysis was performed to differentiate effects of gender, castration, age, breed, treatment and behaviour after surgery. A cox regression was also performed to analyse these same effects in survivability.

Results: A larger proportion of amputee bitches than dogs are associated with cancer while a larger proportion of dogs than bitches are associated with trauma; castrated/spayed dogs were diagnosed older than intact dogs; cancerous causes of amputation were diagnosed later in dogs' lives than traumatic causes; dogs showing an animal behaviour in the first week after amputation are diagnosed with cancer younger than dogs showing a vegetal/rock behaviour. Treatment by chemotherapy has a better survivability than holistic therapy, and patient receiving chemotherapy exhibit animal rather than vegetal/rock behaviour.

Conclusion: The survival analysis gives clear indication of the benefits of chemotherapy over holistic treatments. Animal behaviour post surgery is also related to survivability.

Key words: limb amputation, dogs, epidemiology, survival; surgery;

Several causes have been identified for limb amputation in dogs including trauma, bacterial infections, birth defects and appendicular cancer.

Canine appendicular cancer has been reported 'as an increasingly important disease' (Segal-Eiras et al, 1982). Osteosarcoma accounts for approximately 85% of canine bone tumours (Boerman et al, 2012), being 8-15% of other tumours classed as subcutaneous soft tissue sarcomas (Dennis et al, 2011). The treatment of choice for these conditions is surgical excision by limb amputation (Boerman et al, 1982; Farese et al, 2009), but limb salvage techniques have also been reported (Lascelles et al, 2005). In this survey 71% of the dogs undergoing limb amputation due to cancerous causes underwent chemotherapy, with 29% of them being treated holistically. Around 60% of the dogs diagnosed with osteosarcoma die in the first year after diagnosis with another 10 to 20% dying in the second year (McCleese et al, 2011).

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In the attempt to save their loved pet from the side effects of chemotherapy, owners impelled by compassion, evaluate alternative treatment methods 'with respect for the body's innate ability to heal itself' (Kelleher, 2003). Complications of conventional chemotherapy include toxicological effects like neutropenia, thrombocytopenia and anorexia (Kent et al, 2004) and cardiomyopathy (Moore et al, 2007). Holistic treatments include chiropractic, homeopathy, herbal therapy, acupuncture, special diet and massage (Kelleher, 2003).

Several studies have tried to establish a relationship between animal behaviour and pain, particularly in dogs (Conzemius et al, 1997; Firth and Haldane, 1999; Hansen, 2003), leading to the development of different pain scales (e.g. The Melbourne Pain Scale developed by Firth and Haldane in 1999). It has been proved difficult to correctly assess degree of pain in animals due to the large number of factors involved, and also due to the anthropomorphisation of scales of assessment (Holton et al, 2001). But in all studies it becomes clear that increased mobility and display of a range of normal behaviours is an indication of lower levels of pain.

It is the aim of this research article to analyse the causes of limb amputation in dogs, identifying predisposition factors. A survival prognosis is also conducted on dogs with diagnosed cancer as the cause of the limb amputation. These aspects can be of fundamental importance in helping the veterinary science professional in costumer counselling and decision making with regards to treatment alternatives.

Materials and methods

Questionnaire design and sample

A questionnaire was designed to allow the collection of data to study the population of dogs having a limb amputation. With the aim to study the epidemiological factors leading to amputation several questions were posed, and after data clearing and cleaning with removal of dubious entries and outliers the following variables were selected for analysis:

- Nominal — gender (male, female), castration (en-

Table 1. Parameters of the Cox regression for the survival analysis of n = 65 dogs with limb amputation after cancer diagnosis. The variable 'behaviour on the 1st week after amputation' considers 'animal' and 'vegetal/rock' behaviours. The variable 'treatment' considers the 'chemotherapy' and the 'holistic' practices.							
		ß	SE	Wald Statistic	HR[1] exp(ß)	HR[1] 95% CI[2]	*p*-value
Behaviour	Animal	0					
	Vegetal or Rock	-1.285	0.515	6.224	0.277	[0.101, 0.759]	<0.05
Treatment	Holistic	0					
	Chemotherapy	1.018	0.492	4.285	2.767	[1.056, 7.252]	<0.05
Note: [1]hazard ratio; [2]confidence interval							

tire or spayed/neutered), causes for amputation (trauma, cancer, birth defect, bacterial infection), behaviour of the dog in the first week after amputation (animal, vegetal, rock), type of breed (mixed, working, sporting, terrier, herding, non-sporting, hound)

- Ordinal — body condition score (BCS) before amputation
- Scale — age at amputation (months).

Data were collected in 2010, after a questionnaire directed to all the members of the blog Tripawds.com. This blog contains members from all over the world but mainly from the USA, and from these, 152 members, answered the questionnaire.

Statistical methods

Scale type data were tested for normality with the Kolmogorov-Smirnov test and were found to be normally distributed ($p>0.05$). The prerequisite of homogeneity of variances was also tested and delt accordingly. The analysis of data was performed between the variables listed above, using the following statistical approaches:

- Between nominal variables with the Pearson's chi square, or the Fisher's exact test when prerequisites were not met
- Between nominal variables (used as factors, 2 levels) and scale data with the independent samples t-test, done after the Levine's test for homogeneity of variances.

Follow-up data were not available other than for cancer cause, and therefore a survival analysis for cancerous dogs only was performed, where the dependent variable 'survival time after amputation' was considered. The independent dummy categorical variables considered were: BCS before amputation, behaviour of the dog in the first week post amputation (animal, vegetal/rock) (see below); type of treatment (chemotherapy, holistic); presence of metastasis after treatment (yes, no); type of cancer (lymphoma, carcinoma, sarcoma, unknown, multiple type); gender (male, female); castration (entire, spayed/neutered. The covariates considered were: age at diagnosis (month), and waiting time between diagnosis and surgery (weeks).

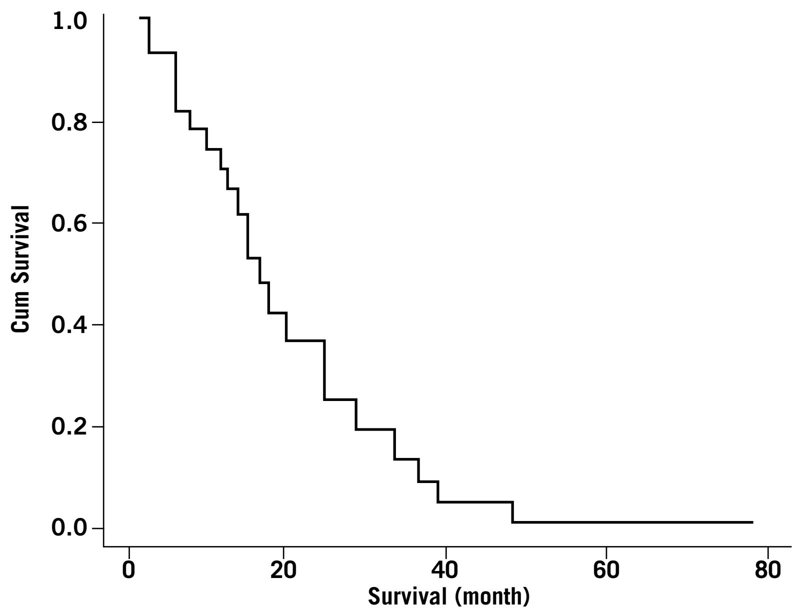

Figure 1. Survival function plot after application of the fitted model. Cumulative survival equals 1.0 immediate after surgery and decreases with time to 0.0, 50 months after surgery when all the dogs are expected to be dead.

Holistic treatment is the use of alternative and complementary therapies such as chiropractic, homeopathy, herbal therapy, acupuncture, special diet and massage. The dog's behaviour was evaluated as rock, vegetal and animal, corresponding respectively to lack of movement and passive behaviour requiring hand feeding; some movement but always lying down, limited response to stimulus but able to feed himself if food presented; able to stand up and move around, looking for food and water and able to regulate intake according to needs, guard attitude and response to stimulus. For convenience of analysis vegetal was merged with rock behaviour, and therefore

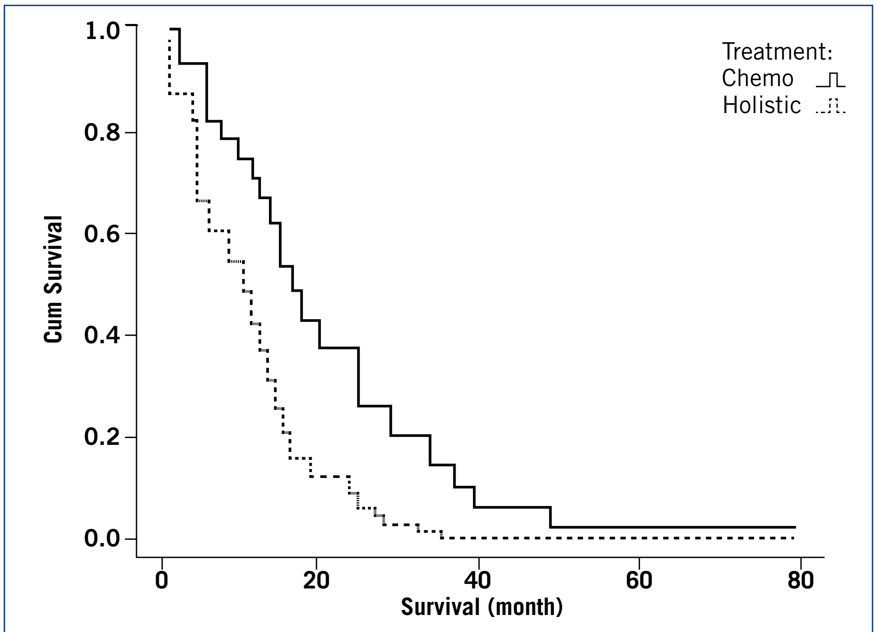

Figure 2. Survival function plot after application of the fitted model. Cumulative survival equals 1.0 immediate after surgery and decreases with time to 0.0, 50 months after surgery in dogs treated by chemotherapy and 35 months after surgery later in those treated holistically.

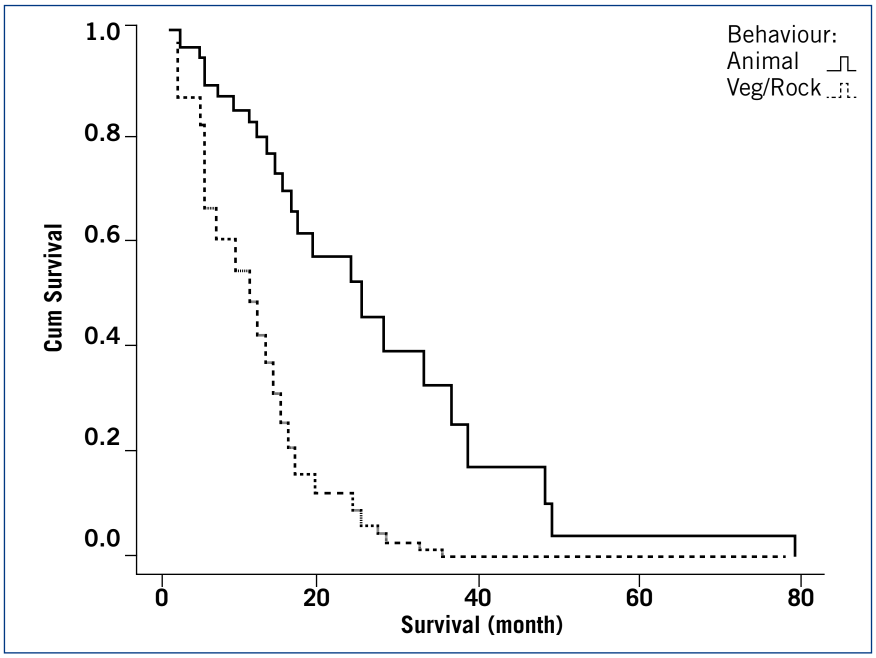

Figure 3. Survival function plot after application of the fitted model. Cumulative survival equals 1.0 immediate after surgery and decreases with time to 0.0, 50 months after surgery in dogs displaying animal behaviour and 35 months after surgery in those displaying vegetal/rock behaviour.

'behaviour of the dog in the first week post amputation' was considered to have two levels. With regards to the type of treatment, mixed treatment was disregarded to avoid confounders. After data cleaning and disregarding of causes other than cancer, n = 65 dogs diagnosed with appendicular cancer were used in this study, where dogs still alive were censored (n = 23, 35.4%)

A multivariable Cox regression analysis was implemented and the final adjusted model was obtained via forward stepwise selection of variables, after the Wald test. The -2 Log Likelihood test was used to evaluate the fitness of the adjusted model. Plots of partial survival curves for the different levels of the significantly different variables were also produced. The significance level was set to $p<0.05$ in all the tests. The analysis was done using the software IBM® SPSS® Statistics 21.

Results

A vast range of dog breeds were included in the amputee analysis; when classed by type, n = 63 (42%) had mixed origin, n = 28 (18 %) were working, n = 32 (21%) sporting, n = 8 (5%) terrier, n = 10 (7%) herding, n = 5 (3%) non-sporting and n = 6 (4%) hound. When classed by size (pure breed only), n = 2 (2%) small, n = 59 (68%) medium and n = 26 (26%) large. With regards to gender, n = 78 (51%) were females and n = 74 (49%) were males. Only n = 7 (5%) of the animals of both gender were not spayed or neutered. With regards to amputee dogs due to cancer, when classed by type, n = 22 (33.8%) had mixed origin, n = 15 (23.1 %) were working, n = 14 (21.5%) sporting, n = 3 (4.4%) terrier, n = 5 (7.7%) herding, n = 3 (4.4%) non-sporting and n = 3 (4.6%) hound. With regards to gender, n = 40 (61.5%) were females and n = 25 (38.5%) were males. The same n = 7 (10.8%) intact dogs of both gender, were counted and therefore all the intact dogs were amputee due to cancer. Using the 3rd edition of the *International Classification of Diseases for Oncology*, n = 1 (1.5%) of the cancers were classified as lymphoma, n = 5 (7.7%) as carcinoma and n = 44 (67.7%) as sarcoma: n = 38 (58.5%) osteosarcoma, n = 5 (7.7%) fibrosarcoma and n = 1 (1.5%) angiosarcoma. Cancers of unknown n = 9 (13.9%) and multiple type n = 10 (15.4%) were present in the sample.

A Pearson's chi square test for goodness of fit was applied to the frequencies of breeds classified by type and size (only pure breed) in all the amputees and the cancerous only and no significant differences ($p>0.05$) were found. Therefore no predisposition of breeds to cancer was made evident.

A Pearson's chi square test for independence using gender (male, female) and causes of amputation (cancer, trauma) found a positive association ($p<0.05$) between female dogs and cancer, suggesting that females are more prone to cancer than males; birth defects and infections were disregarded from this analysis.

A Cox regression model was successfully fitted ($p<$

0.01) with a -2 log likelihood of 115. The significant variables found were 'treatment' (chemotherapy, holistic) ($p<0.05$) and 'dog behaviour in the first week after amputation' (animal, vegetal/rock) ($p<0.05$). Dogs treated by chemotherapy (mean = 23.5; median = 16 month) were seen to have a longer survival time than those treated holistically (mean = 11.1; median = 4.5 month); and dogs showing an animal behaviour in their first week after amputation were also seen to survive longer (mean = 23.2; median = 14 month) than those showing vegetal/rock behaviour (mean = 9.8; median = 8 month). The full description of the model coefficients is summarised in *Table 1*. *Figure 1* plots the survival function at means of variables and *Figures 2* and *3* plot the partial survival functions for the different levels of the variables found significant.

A Fisher's exact test of independence, using behaviour (animal, vegetal/rock) and treatment (holistic, chemotherapy) as nominal variables, have also shown to be significant ($p<0.05$), with dogs displaying animal behaviour being mainly treated by chemotherapy and the others, holistically.

With respect to cause of amputation, significant differences were found for age at diagnosis ($p<0.05$), when considering trauma (mean 3.98 years, 95% confidence interval (3.21; 4.75)) and cancer (7.66, (7.41; 8.01)), and therefore traumatic causes of amputation are diagnosed earlier in the life of dogs and cancerous causes later.

With regards to cancerous dogs only, those showing an animal behaviour (6.17, (5.78; 6.56)) in the first week after amputation are diagnosed younger ($p<0.01$) than dogs showing a vegetal/rock behaviour (7.70, (7.34; 8.06)). Significant differences were also found for spayed/neutered and entire dogs ($p<0.05$). Intact animals tend to have an earlier diagnosis of appendicular cancers (2.79, (0.86; 4.72)) than spayed/neutered animals (7.69, (7.36; 8.02)). These results need to be interpreted with care as the samples are highly unbalanced (58 spayed/neutered against seven only intact), and the intact dogs sample is very small; using the non-parametric Mann-Whitney U test the analysis is not significant ($p>0.05$).

Discussion

With regards to gender of amputee dogs, no significant differences ($p>0.05$) were found for age at diagnosis. An association between females and cancer was found to be significant ($p< 0.05$) when considering the proportion of amputees due to cancer or trauma, with the former having a higher probability in relation to males there is definitively a relation between females and cancer. Misdorp and Hart (1979) reported 30 years ago that sarcomas in females are larger in size than those observed in dogs, however it should be kept in mind that the present study refers to sarcomas in the limbs leading to limb amputation. Anfinsen et al (2011) found no gender predisposition in bone tumours in agreement with Rosenberger et al (2007) and Ru et al (1998), but these authors refer to several others where predisposition in females to osteosarcoma was reported in the Saint Bernards (Brodey and Rise, 1969), and axial osteosarcoma predisposition was reported indiscriminately in females (Heyman et al, 1992); reports indicating a male predisposition to osteosarcomas, arising mainly in limbs, were also found (Brodey and Abt, 1976; Misdorp, 1980). The most conclusive study, however, can be considered as that made by Bonnett et al (2005) and the companion paper Egenvall et al (2005) using a sample of 350 000 dogs where a mortality rate ratio (RR) female/male of 1.1 was reported for tumour and 0.8 for trauma, therefore, favouring predisposition of females to tumours and males to trauma; these results are in complete agreement with those of the present study (3.1 and 0.32 respectively). Note, however, that the other authors' results were related to all causes of death including mammary cancer in females and the present study refers to cases leading to limb amputation. Munro and Thrusfield (2001) found that non-accidental inflicted traumatic injuries are more common in males, justified by the higher degree of aggressiveness in males, resulting in the predisposition of males to trauma leading to limb amputation.

In this study age at diagnosis for amputation has a mean value of 6.9 (6.63; 7.17) years, and 7.16 (6.76; 7.56) years for cancerous dogs, which agrees with Anfinsen et al (2011) and sits between Farese et al (2009) (6.58 years) and Bhandal and Boston (2011) (8.84 years), all referring to dogs with diagnosed osteosarcoma, the first with limb predilection and the former two in the limbs only. It should be noted, however, that the present study includes all types of cancer leading to amputation, with osteosarcoma being the most frequent (58.5%) form of cancer.

The effect of castration on age at diagnosis was found not to be significant ($p>0.05$) when considering all the amputation causes, but was found to be significant in cancerous dogs: neutered/spayed dogs were diagnosed at a later stage in their lives (7.69, (7.36; 8.02) years) than intact dogs (2.70, (0.86; 4.72) years). The decrease of incidence of mammary gland related neoplasia, in older dogs is well documented (Root-Kustritz, 2007). Several studies have also shown an increased risk of osteosarcoma in neutered dogs, especially when this procedure is performed at an early age (Ru et al, 1998; Cooley et al, 2002; Root-Kustritz, 2007). This is not in agreement with

Key Points

- The bitches have predisposition for cancer while dogs have a predisposition for trauma. This aspect can be due to the higher level of endocrinal activity in females and territorial behaviours in dogs.
- Castrated/spayed dogs were diagnosed with cancer older, than intact dogs, possibly due to the reduction in endocrinal activity which also impacts negatively on the onset of territorial behaviour in males.
- Cancerous causes of amputation were diagnosed later in dogs' lives, than traumatic causes, which can be explained by the inexperience of younger animals leading to accident, resulting in trauma.
- Dogs showing an animal behaviour in the first week after amputation are diagnosed with cancer younger than dogs showing a vegetal/rock behaviour, and therefore younger animals reacted better to amputation, and also showed higher survival rates.
- Treatment by chemotherapy has a better survivability than holistic therapy and should be preferred when the survivability is likely to be higher, such as in younger animals.

the authors' findings where the opposite was found. Although, as indicated previously, these findings are reported with reservations.

With regards to the age at diagnosis, significant differences were found between cancerous (7.66, (7.41; 8.01) years) and traumatic causes (3.98, (3.21; 4.75) years), and therefore cancerous causes of limb amputation happen later in the dogs' life than traumatic causes. This result makes sense, as it can be expected for cancer to show up more frequently in advanced age, and accidents to happen in younger inexperienced animal. Munro and Thrusfield (2001) indicated that younger animals have a higher predisposition for non-accidental inflicted traumatic injuries than older animals; and the conclusive study made by Bonnett et al (2005) and Egenvall et al (2005) also reported that predisposition to traumatic death decreases with age while predisposition to cancer increases with age.

Survival analysis of dogs diagnosed with cancer, revealed that treatment ($p<0.05$) and behaviour ($p<0.05$) were significant factors. Dogs treated with chemotherapy (median 16.0 month, 95%CI (10.5; 21.5)) have been shown to survive longer than dogs treated holistically (4.5 (0.7; 8.3)). Dogs showing animal behaviour in the first week post amputation (14.0 (7.5; 20.5)) have been shown to survive longer than dogs showing vegetal/rock behaviour in the first week (8.0 (4.1; 11.9)).

Treatment with chemotherapy (carboplatin, cisplatin, and doxorubicin) has been shown previously to double survivability in dogs after surgical removal of a limb due to osteosarcoma (Coomer et al, 1996) in single agent protocols (Berg, 1996; Phillips et al, 2009) and in protocols using two agents (Chun et al, 2000; Kent et al, 2004). Carboplatin was the drug of choice in the sample used in this study, in single drug protocol or in combination with doxorubicin, some dogs were also treated with gemcitabine and lomustine. The results of this study are, therefore, not surprising.

With regards to the behaviour in the first week after amputation surgery, the results are also as expected. A dog developing an animal behaviour, showing mobility and general activity as opposed to daytime sleeping, indicates the presence a lower degree of pain and discomfort (Wiseman et al, 2001) which, in this study is found to be associated with higher survival rates. The significant ($p<0.05$) association between treatment by chemotherapy and animal behaviour is just a reiteration of the survival analysis.

Dogs showing an animal behaviour (mean 6.17 years 95%CI (5.78; 6.56)) have also been shown to be diagnosed significantly ($p<0.01$) younger than dogs showing a vegetal/rock behaviour (7.70 (7.34; 8.06)), and despite the fact that age at diagnosis was found not to be significant in the survival analysis, younger amputees can be expected to survive longer; it is likely these cases were diagnosed at an early stage of the cancer.

Several authors (Bonnett et al, 2005; Egenvall et al, 2005) have identified a relationship between large dog breeds and development of osteosarcoma. The present study did not identify any breed effect, however it shall be noted that this study refers to all types of cancer leading to limb amputation.

Conclusion

In conclusion this study found an association between amputee bitches and cancer and amputee dogs and trauma. Castrated/spayed dogs were diagnosed for amputation later in their lives, when compared with intact animals; cancerous causes were also diagnosed later in their lives, when compared to traumatic causes leading to amputation. The survival analysis of amputee cancerous dogs showed an advantage of treatment by chemotherapy over holistic approaches and of animal over vegetal/rock behaviour in the first week after amputation. An association was also found between animal behaviour and treatment by chemotherapy which reiterates the survival analysis results. This information can be used by practitioners in decision making with respect to which treatment strategies to adopt; it is therefore also important information to be used in owner counselling. **VN**

Acknowledgments and conflict of interests
I would like to acknowledge the sympathy of the tripawd.com blog administrators, as well as its members, for allowing the use of the data they collected in the 2010 dog limb amputation survey, on the production of this paper. The author has no conflict of inter-

ests to report.

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Mastitis vaccination in dairy cattle: a meta-analysis of field case-control trials

Vacinação contra a mastite em vacas leiteiras: uma metanálise de ensaios clínicos de campo com controlo

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Resumo: A mastite é a primeira causa de perdas económicas no sector leiteiro. A eficácia das vacinas existentes contra a mastite é questionada por diversos autores. É o objectivo deste estudo, contribuir para o esclarecimento desta questão. Uma metanálise foi efectuada em 15 ensaios clínicos de campo, incluindo um total de 7941 vacas (4317 vacinadas e 3624 usadas como control). Um modelo de efeitos aliatórios foi ajustado com uma significância de P<0,01. A unidade considerada foi o rácio (entre vacas vacinadas e usadas como controlo) da razão entre vacas com mastite e normais. Este foi calculado como tendo um valor de 0,604 com um intervalo de confiança a 95% de [0,373; 0,979] depois da correcção do enviezamento de publicação. Conclui-se que a vacinação para o controlo de mastites traz alguns resultados positivos, mas a vantagem económica do seu uso depende do balanço entre custos e benefícios. Como tal, as medidas preventivas tradicionais continuam a desempenhar um papel fundamental no controlo da mastite; a vacina contra a mastite pode ser utilizada como um complemento no combate à mastite, mas não como um substituto de todas as outras medidas preventivas.

Palavras-chave: mastite, mamite, vacas leiteiras, vacina, metanálise

Summary: Mastitis is the first cause of economic loss in the dairy sector. The efficacy of the existing vaccines against mastitis is questioned by several authors. It is the aim of this paper to shed some light on this question. A meta-analysis was conducted on 15 field research trials, which included a total of 7941 cows (4317 vaccinated and 3624 used as control). A random-effects model was adjusted and found significant (P<0,01). The unit used was the rate ratio between vaccinated cows and controls and was found to have a value of 0,604 with a 95% confidence interval of [0,373; 0,979], after correction for publication bias. It was concluded that vaccination to control mastitis has a slight advantage, but the economics of its use depends on the weighting between its cost and its benefit, and therefore traditional preventive measures still have an important role to play; Mastitis vaccines can be used as a complement of all the other preventive measures but not as a substitute of these.

Keywords: mastitis, dairy cow, vaccine, meta-analysis

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Introduction

Mastitis is the first cause of economic loss by disease in dairy cattle in developed countries worldwide (Nielsen *et al.*, 2010). These include losses in actual and potential production, shortening of productive life, losses in milk quality, medicine costs, costs of veterinary care (Hogeveen *et al*., 2011) and potential implications in the general health of the animal including reproductive performance (Dobson *et al*., 2008). Mastitis is characterised by an inflammation in one or more quarters of the udder and can have an infectious or a traumatic origin (Kudi *et al*., 2009). The most frequent agents of infection in dairy cattle are *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus uberis* and *Streptococcus dysgalactiae* (Schukken and Kremer, 2001). Mastitis caused by the first of these agents is classified as contagious, and by the others environmental (Tyler and Ensminger, 2006).

The research into vaccines against *Staphylococcus aureus* started in the early 1990s (Michie, 2002), and with regards to mastitis, several vaccines have been tested and advances have been made (e.g. González *et al*., 1989) but there are doubts with regards to the efficacy of the commercially available vaccines (Leitner *et al*., 2003). There are several types of vaccines that have been tested, and with regards to *S. aureus* these include whole organism (cellular lysates, inactive and attenuated vaccines) and sub-units (toxins, surface proteins and polysaccharides) (Wallemacq, 2010). Mono and polyvalent vaccines have been tested: *S. aureus, E. coli, S. aureus / E. coli, S. aureus / Streptocccci* (e.g. Yoshida *et al*., 1984; Gonzalez *et al*., 1989; Giraudo *et al*., 1997; March *et al*., 2010); these were tested in adult cows exposed before to the disease and in heifers not exposed to the disease (e.g. Calzolari *et al*., 1997, Moromoto *et al*., 2011); and also different inoculation protocols were followed using, none, one or two boosters (e.g. Nordhaug *et al*., 1994; Watson *et al*., 1996).

The difficulties with the development of a vaccine against mastitis were identified a long time ago; Mellenberger (1977) states that these difficulties are related to the large number of different organisms involved in the pathology and with the fact that when the pathogen remains in the duct and alveoli as subclinical infection, it is not attacked by the humoral antibodies, which happens only in clinical mastitis cases, when the pathogens penetrate the mucous membrane. Denis *et al*. (2011) who has recently done a review of the state of the art in mastitis vaccination, recognises the doubts regarding the efficacy of the existing vaccines and points several potential directions to follow in future developments; these authors conclude that the design of an effective vaccine is challenging, as the immune response in the mammary gland is unique. Bovine mastitis is an evolving disease and different pathogens have shown to have different levels of importance in the development of the disease through time (Bradley, 2002).

The aim of this study is to make a meta-analysis of existing clinical case-control trials, to bring to light important knowledge regarding the efficacy of the mastitis vaccines.

Material and methods

The literature search was extensively performed with the use of Google scholar for English, French, Spanish and the Portuguese languages: http://scholar.google.co.uk, http://scholar.google.fr, http://scholar.google.es, http://scholar.google.com.br, and also all the databases assessed by the University of the West of England library search engine of the Animal and Land sciences subject, which include: CAB Abstracts, Cambridge Journals Online, Directory of Open Access Journals, EBSCO Host Electronic Journals Service, Emerald, MEDLINE, Nexis, PubMed, SAGE Journals Online, ScienceDirect, Taylor & Francis, Willey Online Library, UWE e-journals at OVID and Zetoc. Simple and multiple combinations of the following key words were translated into the languages considered and used in the search: mastitis, vaccine, trial, case control, cow, dairy, cattle, and immunisation. Inclusion and exclusion criteria were defined as follows: Animals were considered to have mastitis when clinical mastitis was identified; some trials report their results considering number of quarters infected, which is impossible to convert into number of animals infected and were, therefore, excluded; the minimum period of observations considered after vaccination was 3 months, but no maximum was considered; experimental trials where the animals were challenged with pathogens were not considered; only independent studies without conflict of interests were considered.

The outcome measure considered in the analysis was the logarithm of the risk ratio (RR). The residual heterogeneity (τ^2) was estimated with the restricted maximum-likelihood estimator and found to be 0,576 with a Wald 95% confidence interval of [0,249; 2,088]. The homogeneity of the data was tested with the Cochran's Q-test, and was found to be significant (Q=80,32, df=14, P<0,001). As the data was shown to be heterogeneous an initial mixed effects model was used. The covariate moderators used were: "absolute latitude of the location of the trial" (to add for climatic effect); "year of publication of the communication" (to add for genetic and technological gains); and "duration" (to add for the temporal effect due to different durations of the trials). The factor moderators used were: "vaccine agent" (*Staphylococcus aureus*, *Escherichia coli*, and multiple), "booster" (no booster, 1 or 2) and "exposed" (trials using heifers never exposed to mastitis or undifferentiated age pre exposed). As none of the moderators was found to be significant (P>0,05), a random effects model was finally used in detriment of a fixed effects model to add for the heterogeneity observed. After the random effects model a radial plot was produced to analyse the sensitivity of the model by looking at the source of heterogeneity and the extent of heterogeneity due to each study. The publication bias was evaluated via a funnel plot and tested via a regression test (weighted regression with multiplicative dispersion). The normality assumption was evaluated via Q-Q normal plot, and as seen in Figure 1, the assumption can be made once all the studies fall in the pseudo confidence envelop.

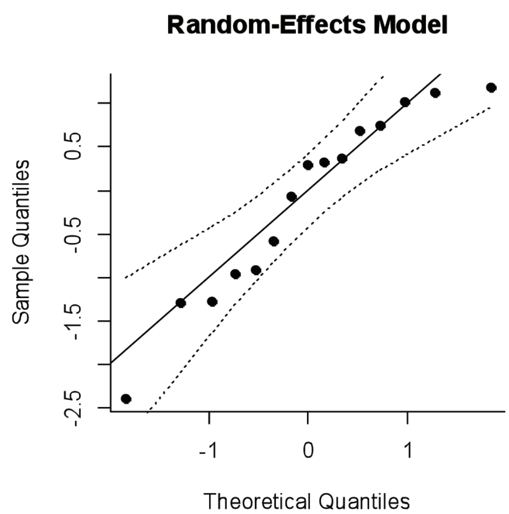

Figure 1 – Q-Q plot and respective 95% pseudo confidence envelope for assumption of normality after the random effects model.

The statistical analysis was performed with the freeware R CRAN for Windows® version 2.15.0. platform x86_64-pc-mingw32/x64 (64-bit) (Comprehensive R Archive Network , http://cran.r-project.org/). The specific meta-analysis package "metaphor" (author: Wolfgang Viechtbauer) was used.

Results

The meta-analysis was performed with 15 studies (Table 1) that met the criteria set in the methodology. These studies took place between 1984 and 2011 in several places around the world. A total of 7941 cows were used in the trials, 4317 were vaccinated and 3624 were used as controls.

The outcome measure considered in this study was the relative risk (RR) or risk ratio and is calculated as risk if vaccinated divided by the risk if not vaccinated. The random effects model adjusted was found to be significant (P<0,01) and the estimated log value is -0,644 [-0,207; -1,082] of the RR that after exponentiation gives the 0,50 [0,34; 0,81] value observed in the forest plot (Figure 2). The model is estimated to have a total amount of heterogeneity of τ^2 = 0,576 [0,249; 2,088], and the percentage of total variability due to heterogeneity is I^2 = 91,7% [82,9; 97,3]. This heterogeneity was found to be significant after the Q test (P<0,001).

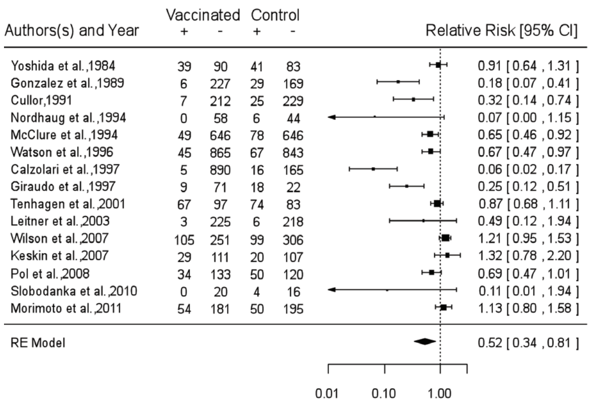

Figure 2 – Forest plot showing the results of the 15 studies examining the effectiveness of the mastitis vaccine. The relative risk ratio (RR) of mastitis infection favours the vaccine if lower than 1. The RR are presented with 95% confidence intervals, and are based on the adjusted random effects (RE) model.

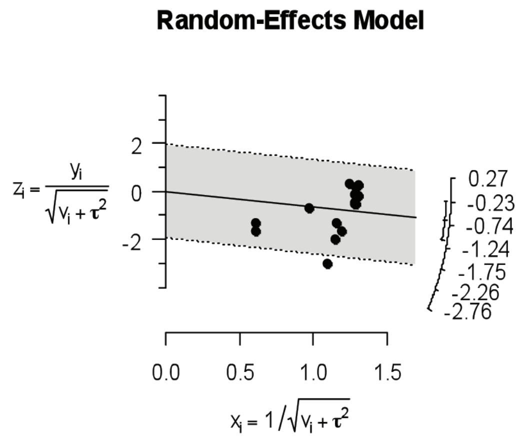

Figure 3 – Radial plot for the adjusted random effects model.

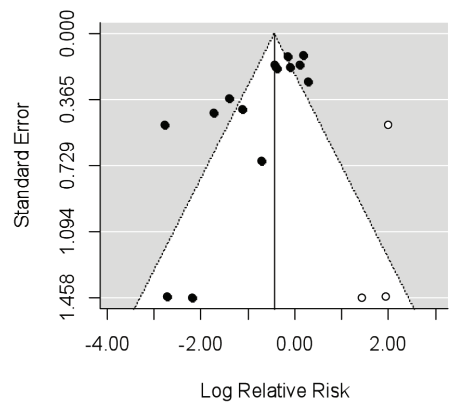

Figure 4 – Trim and fill funnel plot, highlighting 3 missing studies and a publication bias.

Table 1 – The 15 studies considered in this meta-analysis and the variables used as moderators. The number of vaccinated and control cows, together with the number of observed negative and positive to mastitis can be found in Figure 1.

Trial	# cows	Duration (month)	Vaccine agent	# boost	exposed	absolute latitude
Yoshida *et al*., 1984	253	6	*S.aureus*	1	yes	33º
Gonzalez *et al*., 1989	431	18	*E. coli*	0	no	38º
Cullor, 1991	473	10	*E. coli*	2	yes	39º
Nordhaug *et al*., 1994	108	10	*S.aureus*	0	no	60º
Mc Clure *et al*., 1994	1419	5	*E. coli*	1	yes	33º
Watson *et al*., 1996	1820	10	*S.aureus*	1	yes	37º
Calzolari *et al*., 1997	1076	4	Multi	1	no	31º
Giraudo *et al*., 1997	120	9	Multi	1	no	33º
Tenhagen *et al*., 2001	321	3	*S.aureus*	1	yes	38º
Leitner *et al*., 2003	452	10	*S.aureus*	2	no	32º
Wilson *et al*., 2007	761	20	*E. coli*	1	yes	42º
Keskin *et al*., 2007	267	7	Multi	1	yes	40º
Pol *et al*., 2008	337	7	*E. coli*	1	yes	32º
Slobodanka *et al*., 2010	40	8	*S.aureus*	0	yes	45º
Morimoto *et al*., 2011	480	10	*E. coli*	1	yes	34º
Total	7941					

The amount of overall heterogeneity due to each study is evaluated with the radial plot shown in Figure 3, and as can be observed all the studies considered fall in the confidence envelop, with a small exception. The RR of the different studies, having different precisions, show therefore to be consistent. This plot also shows some evidence of existence of publication bias, which can be even better observed through the trim and fill funnel plot (Figure 4), with the number of missing studies being estimated as 3, being 2 of them in the right hand bottom corner of the plot. This positioning gives evidence of small trials publication missing when results obtained contradict the expected positive effect of the vaccine. The rank correlation test for asymmetry (Kendall's T) was found to be significant (P<0,05), and therefore a publication bias was found present. The new Overall RR after fill and trim for correction of asymmetry is 0,60 with a 95% CI of [0,37; 0,98].

Discussion

The history of mastitis vaccine is connected with the search for protection against the microorganisms causing it, and therefore started in the beginning of the last century (Michie, 2002; Wilson and González, 2003). The search of a bacterin to combat environmental pathogens (mainly *Escherichia coli*, but also *Klebsiella* spp, *Enterobacter* spp and *Pseudomonas* spp) is the aim of researchers since 1910, but not until the late 1980s was some efficacy reported (Wilson and González, 2003). The main antigen bacterin used for the production of anti-coliform vaccines is known as J5, but RE-17 mutant *Samonella typhimurium* bacterin toxicoid is also used. There are several of these vaccines available on the market, such as Pfizer Animal Health, Upjohn J-5 Bacterin®; Bayer Mastiguard™; Merial, J-Vac®; IMMVAC Endovac-Dairy®; Novartis Animal Health J-5 Shield™.

The vaccination against *Staphylococcus aureus* has also been attempted from the beginning of last century, including attenuated, fixed or lysed organisms. Also in the 1960s polysaccharides from the capsule were used, and later in the 1970s enterotoxins were also used as antigenic components (Michie, 2002). More recently experimental DNA recombinant protein and recombinant protein alone vaccines are being tested (Denis *et al.*, 2011). In present times one vaccine specific for *S. aureus* mastitis is available in the market, namely Boehringer Ingelheim, Lysigin® Somato-Staph®. Since the 1980s attempts have also been made to design a vaccine against *Streptococcus uberis* but this is still not available commercially (Denis *et al.*, 2011).

HIPRA, Startvac®, is a vaccine commercially available and fights both *E. coli* and *S. aureus* through inactivated *E. coli* J5 and inactivated *S. aureus* (CP8) SP 140 strains.

From these vaccines, Startvac®, is the only one used in the UK and all the others are used in the USA. The meta-analysis did not identify any significant moderator, including the vaccine agent (including *E. coli*, *S. aureus* and multi agent), and therefore it was not found that a mixture of agents in the vaccine would improve its efficiency. Accordingly to the results of this study, all these types of vaccines will have the same degree of efficiency, and therefore the choice (where possible) should be done having in mind the application protocol and the final price for following the protocol.

The RR after fill and trim for correction of asymmetry was found to be 0,60 with a 95% CI of [0,37; 0,98], showing the exclusion of the value 1 in the CI and therefore an advantage on the use of vaccines against mastitis. Considering, however, the upper limit of the CI, full advantage is not completely evident when considering the cost of the vaccines.

The efficacy achieved so far with the vaccines existing in the market is still relatively low, and therefore ongoing discussions are taking place between scientists regarding the best strategy, while experimentation is also giving new clues about how to defeat mastitis (e.g. Leigh *et al.*, 2010). Transgenic resistant cows have already been engineered, capable of expressing an antibacterial endopeptidase in their mammary glands, which specifically targets the cell wall of *S. aureus* (Rainard, 2005). Sordillo (2011) summarised some of the reviews done in the past decade and highlights some directions that research is taking to explore ways of improving vaccination efficiency: for example the need to develop protocols that enhance mucosal cellular immunity and trafficking of memory T cells to the mammary gland as a strategy to develop effective immunization; and the use of marker assisted technology to identify genes and chromosomal regions of interest. DNA vaccination is a recent strategy, where plasmids are introduced in the animal to lead to the activation of both humoral and cellular immune responses to the antigen (Talbot and Lacasse, 2005).

Other moderators considered in the model were the absolute latitude to test an eventual effect of climate; year to test the evolution of the technology in the 27 years spread of trials used; the duration of the trial to consider the time effect, the number of boosters and the use of heifers or previously exposed groups of animals, but none of these was found to be significant. The moderator year is also not significant and therefore there is no evidence of developments in the efficacy of the vaccines used today when compared with those used 25 years ago.

This study shows an advantage on the use of vaccines to control mastitis that is not completely conclusive and agrees with the conclusions of the majority of independent authors writing about mastitis vaccination efficiency. A mastitis rate ratio for vaccinated versus control cows of 0,604 after correction for asymmetry, favours the vaccination, but with a 95%CI of [0,373; 0,979], this advantage is small. The advantage of the use of mastitis vaccines needs to be weighed between the costs of not using them with the costs of using them, but the use of the traditional preventive measures are still of fundamental importance. As indication Nielsen *et al.* (2010) has calculated the cost of a clinical mastitis case in Sweden in €278 when considering withdrawn of milk, lower prices due to high somatic cell counts, veterinary fees, drugs and increased risk due to recurrent cases; extra labour was not considered. The cost of a full vaccination programme with boosters and conferring protection for 1 year was reported to be between £7 and £9 by Balsom (2011).

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Publication six

Mata, F., Lam, A. (2013) Investigating the relationship between feed and helminthic burden of captive birds of prey in Hong Kong. *Zoo Biology*. 32 (6), pp.652-654. DOI: 10.1002/zoo.21103

Zoo Biology 32: 652–654 (2013)

BRIEF REPORT

Investigating the Relationship Between Feed and Helminthic Burden of Captive Birds of Prey in Hong Kong

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The life cycle of most parasitic helminthes is related to their hosts feeding habits. Thus we need to investigate the impact of diet on the host's helminthic parasite burden. Not many studies in captive raptors have been conducted and published regarding parasitic infections. The aim of this study is to evaluate the helminthic burden of raptors kept in captivity and establish a relationship with the feed provided. A total of $N = 51$ different species of captive birds of prey were fed different diets consisting in different combinations of day old chicks, chicken breast, whole chicken carcass and mice. Their feces were sampled and the parasite burden was determined. A negative binomial model was successfully fitted to the data and the feeds "mice" ($P < 0.001$) and "whole chicken carcass" ($P < 0.001$) significantly contributed to an increase in the observed burden. Significant differences were also found between species ($P < 0.001$). Raptors fed adult animal carcasses and offal may explain the increase in the observed burden as these feeds have a larger probability of being contaminated by a larger variety of helminthic fauna. Zoo Biol. 32:652–654, 2013.

Keywords: helminthes; fecal egg counts; raptor; feed; Hong Kong

INTRODUCTION

Parasitic diseases are one of the most common causes of morbidity in both captive and wild raptors [Santos et al., 2011]. A study done in Beijing, China, by Zhang et al. [2008], reported the infection rate of nematodes in raptors dying in a Rescue Centre to be 44%, with the majority found in the intestines, oral cavity, stomach and esophagus. Trematodes, cestodes, acanthocephalans, and digenea are common helminthes found in raptors [Sanmartin et al., 2004; Santoro et al., 2010; Santos et al., 2011]. Santoro et al. [2010] reported levels of helminthic infestation in Italian wild raptors reaching 95%, with pathological changes associated reaching 74% of the birds. Filarioid nematode infection is an example of a nematode condition capable of leading to emaciation, depression, diarrhea, and death in wild raptors [Larrat et al., 2012] and has been reported in wild birds of prey [Sanmartin et al., 2004; Santoro et al., 2010].

The codes of good husbandry practice highlight the need to maintain an appropriate health and welfare status of animals kept in captivity [Heidenreich, 1997]. In order to maintain a healthy status of captive animals, a good knowledge of possible diseases, treatment and methods of prevention are essential [Heidenreich, 1997]. Despite the fact that more raptors are introduced to zoos and wildlife parks, there is limited research relating to captive raptors' endoparasites and their sources [Sanmartin et al., 2004; Santoro et al., 2010].

METHODS

This study was conducted at the Veterinary Hospital of Kadoorie Farm and Botanic Garden (KFBG) in Hong Kong

Conflicts of interest: None.

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and a total $N = 51$ birds were used: $n = 15$ owl (collared scops owl, *Otus lettia*; barred owl, *Strix varia*; barn owl *Tyto alba*; and brown fish owl, *Bubo zeylonensis*), $n = 9$ falcon (peregrine falcon, *Falco peregrinus*), $n = 10$ hawk (Crested goshawk, *Accipiter trivirgatus*; common buzzard, *Buteo buteo*, and black baza *Aviceda leuphotes*), $n = 11$ kite (black eared kite, *Milvus migrans*) and $n = 6$ eagle (white bellied sea eagle, *Haliaeetus leucogaster*; and crested serpent eagle, *Spilornis cheela*).

The variables considered were "species," "diet" and "fecal nematode egg counts;" other variables like "age," "weight," and "gender" were not considered as these would have been confounded by the effect of species'. The diet of the different animals was recorded and consisted of "mice," "whole chicken carcasses," "day old chicks," and "chicken breast" in different arrangements and quantities, depending on species and individuals. Mice are culled animals from a bioterium, day old chicks are culled males of laying breeds after sexing with 2–3 days of age and chicken carcass and breasts are obtained in a poultry slaughterhouse from carcasses ungraded for human consumption. All these are obtained in a daily basis, fresh, being the raptors occasionally fed with frozen feed (normally fresh feed is not available in weekends and bank holidays). According to the operational guidelines for wild animal rehabilitation of KFBG [2009], raptor should be fed a quantity equivalent to 10% of their live weight per day. In the KFBG raptors are dewormed every 6 months with fenbendazole given orally (50 mg/kg).

Microscopic examination of fresh fecal sample is the cornerstone of detection of helminthes [Lavoie et al., 1999] and, therefore, fresh and non-preserved sample were used for examination. Due to the fact that intestinal parasites are shed intermittently, multiple fecal samples must be collected from each individual raptor [Sanmartin et al., 2004]. Individual fecal samples were collected within a week long period from the floor bedding during the daily routine of cage cleaning and feeding. In order to maximize the reliability of the experiment, the whole process was repeated after 1 week, and the fecal count considered was the average between these two collections. Collection was done 5 months after deworming, therefore 1 month before the following deworming.

Sterilized spatulas were used to collect the fecal matter of the bowel evacuation and the white uric acid was separated from the fecal matter to analyze. Each sample was stored in a micro tube and labeled. All samples were stored in a cooler bag until analyzed and the analysis of feces was conducted within 12 hr after collection as recommended by Ballweber [2001]. The McMaster technique was used to count the worm eggs, with flotation induced by a sodium nitrate solution as recommended by Rupley [1997], Kassai [1999], Ballweber [2001], and Krone [2007].

The statistical analysis was performed using generalized linear models of the Poison family to model counts. The data were found to be over dispersed, through the Lagrange multiplier test $P < 0.001$, and therefore the negative binomial model was found to be the best fit. A backwards stepwise method of selection of variables was implemented for selection of those found to be significant at a level of $P < 0.05$; the analysis was performed via the generalized linear modules routine of the software IBM® SPSS Statistics 19.

RESULTS

The negative binomial model was found to be significant after the likelihood chi-squared test ($P < 0.001$) with a deviance of 390. The variable "species" ($P < 0.001$) was found significant together with the diet variables "mice" ($P < 0.001$) and "carcass" ($P < 0.001$); an intercept was also found significant ($P < 0.001$); and the diet variables "chicks" and "chicken breast" were found not significant ($P > 0.05$). The full description of the model, with parameters and respective 95% confidence intervals is found in Table 1. When "mice" is not part of the diet there is a decrease in the number of helminthic egg counts (odd rate lower than 1), which is also the case when "whole carcass" is fed. The

TABLE 1. Negative binomial regression parameters (β) with log link function, for the prediction of the "helminthic egg counts," function of "species" and diet "whole chicken carcass" and "mice"

Variables	β	SE (β)	P-value	95% CI (β)	e^{β}	95% CI e^{β}
Intercept	8.122	0.408	<0.001	7.321; 8.922	3366.667	1512.3; 7494.7
Species			<0.001			
Owl	2.453	0.943		0.605; 4.302	11.627	1.831; 73.854
Falcon	1.907	0.943		0.058; 3.756	6.731	1.060; 42.759
Hawk	2.318	0.937		0.481; 4.155	10.158	1.618; 63.782
Kite	0.121	0.817		−1.479; 1.722	1.129	0.228; 5.593
Eagle	0				1	
Mice			<0.001			
Yes	0				1	
No	−4.206	0.943		−6.055; −2.357	0.015	0.002; 0.095
Carcass			<0.001		1	
Yes	0					
No	−3.025	0.782		−4.559; −1.492	0.049	0.010; 0.225

SE, standard error; CI, confidence interval; the adjusted final model has a deviance of 390; the model has a dispersion coefficient of 1.

TABLE 2. Expected counts of helminthic eggs per gram of feces function of species and diet after application of the fitted model

Species	Diet	Count
Owl	Mice + chick	1,901
	Whole chicken carcass	584
Falcon	Mice + chick	1,101
Hawk	Mice + chicken breast	1,661
Kite	Mice + chick	185
	Whole chicken carcass + mice	3,800
Eagle	Whole chicken carcass + mice	3,368

number of helminthic egg counts for each combination of species and diet, expected to be found after application of the model are presented in Table 2.

DISCUSSION

The main factors responsible for variation of helminthic faunas in birds are correlated with the variety of the diet and the vagility of the bird [Santoro et al., 2012a,b]. Birds with a wider hunting area and with wider distribution will increase their probability to contact and being infected with a wider range of parasites; the variety of the diet also exposes the bird to a wider range of intermediary hosts, therefore widening the probability of encounters with different parasites. Several authors cited by Santoro et al. [2012a,b] refer to the aquatic birds as the most exposed to the richest helminthic communities [e.g., Poulin, 1997].

Sanmartin et al. [2004] studied several helminthic infestations in different species of raptor in Galicia, Spain and found that the buzzard (*B. buteo*) was the raptor with the richest variety of helminthic parasites, concluding that this fact was a reflection of the bird varied diet of invertebrates and small vertebrates. In general Sanmartin et al. [2004] found that falconiforms have a richer diet than strigiforms with these preying mainly on small rodents, and therefore strigiforms' helminthes are limited to a subset of those found in falconiforms, which is also in agreement with the findings of Iriarte et al. [1990]. Su and Fei [2004] referring to goshawks in Taiwan, enumerate different types of paratenic hosts for helminthes identified in these birds: reptiles, amphibians, small birds, small insectivorous mammals and rodents. It is, therefore, evident that variations between species are normal and expected.

The interpretation of parasite burden results obtained through fecal analysis needs to be done carefully. The relationship between egg counts and worm burdens may be influenced by several variables, including "the number of adult worms in the gastro intestinal tract, worm age, host immunity, host age, host sex, stages of infection, fecundity of the worm, feed composition and consistency of the feces and time of day of collecting the feces" [Permin and Hansen, 1998]. On the other hand if the collection conditions are similar, these counts can be useful to compare birds.

CONCLUSION

The results shown by this study clearly show that birds fed with mice and whole chicken carcass show a higher parasite burden, which may be explained by the fact that these feeds have a higher probability of being contaminated with helminthes than breast fillet or day old chicks; explained by the presence of offal. Day old chicks are also fed entire with offal, but the probability of infection is lower once they have a day of live. Clearly raptors fed both entire chicken carcass and mice (kite and eagle) have the highest burden.

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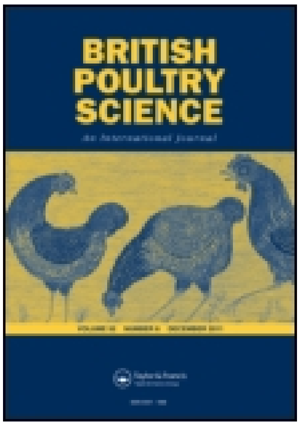

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Comparative mortality and predation in relation to egg production traits of Rhode Island Red, Black Australorp and Hyblack laying hens in scavenging production systems of rural Malawi

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Comparative mortality and predation in relation to egg production traits of Rhode Island Red, Black Australorp and Hyblack laying hens in scavenging production systems of rural Malawi

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Abstract 1. Black Australorp (BA), Rhode Island Red (RIR), and Hyblack (HB) birds were used in farm and field scavenging systems in Malawi, to study mortality through disease and predation in relation to laying performance.

2. Predation was higher in BA than HB.

3. Mortality through disease was higher in RIR than BA and HB.

4. Crossbred HB birds show the lowest combination of mortality and predation, suggesting a heterosis effect.

5. Mortality did not differ on farms and in field environments, suggesting an inability to improve biosecurity in farm conditions.

6. There was a positive relationship between eggshell strength and mortality. Calcium depletion from the birds' bones, limiting foraging and escaping ability may be the explanation, which ultimately increases susceptibility to disease and predation.

INTRODUCTION

The average intake of animal protein (6 kg/capita/year) in Malawi is half of the African average (Gondwe *et al.*, 2003). Poultry livestock, and particularly chickens, play a leading role in the Malawi diet (Safalaoh, 1992; Lwesya *et al.*, 2004), accounting to 75 to 80% of the animal protein (excluding fish) ingested by locals in rural areas (Kampeni, 2001). Also, eggs can be produced easily on a domestic scale and are a much less perishable food than meat alternatives (Ahlers *et al.*, 2009). Poultry are kept in Malawi for either eggs or meat production (Gondwe and Wollny, 2005), and as a source of food and income (Gondwe *et al.*, 2005; Ahlers *et al.*, 2009).

Egg production is not growing at the same pace as population needs, because of the poor quality of feed stuffs available for layers and also the lack of high performing laying breeds adapted to the production systems (Kampeni, 2001). Poor disease control measures have resulted in high chick mortality (Gondwe *et al.*, 1999; Kampeni, 2001). Major constraints in poultry production in Malawi include Newcastle disease (Kampeni, 2001), poor and slow growth rate due to inadequate feed resources (Guèye, 1998; Gaussi *et al.*, 2004; Lwesya *et al.*, 2004), high chick mortality (Guèye, 1998; Kampeni, 2001), and predation (Kampeni, 2000). Chickens in rural Malawi usually survive by scavenging on house waste, plant shoots, seeds, fallen feed grain, and invertebrates (Farrell *et al.*, 2000; Kampeni, 2001; Gondwe and Wollny, 2007). Native chicken breeds show very good adaptation to local scavenging production systems, whether this is due to resistance to disease (Minga *et al.*, 2004; Ahlers *et al.*, 2009), or the capability to use feeds of lower quality (Farrell *et al.*, 2000). However, they have slow growth rates and low

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rates of egg production (Safalaoh, 1997). On average a hen of a local breed lays three clutches of twelve egg per year (Safalaoh and Sankhulani, 2004).

Local breeds including Yakuda, Yofira, Yoyela, Chiphulutsa and Kawangi, dominate the genetic pool available (Gondwe *et al.*, 2003). The Black Australorp (BA), a dual purpose fowl (Roberts, 1997), tend to be broody but calm and docile (Kent and Compton, 2011). BA were introduced in Malawi from the early 1960s to crossbreed with local breeds, aiming for performance improvement (Kampeni, 2001; Gondwe and Wollny, 2003), and because it adapts well to the local conditions (Safalaoh, 2001). The Rhode Island Red (RIR) is a multipurpose breed (Roberts, 1997) also common and after recent introduction to Malawi (Gondwe *et al.*, 2003) and is very popular in Africa (Kekeocha, 1984). The cross between BA and RIR, named Hyblack (HB) in Malawi, performs well under local conditions. It has been bred for hardiness and adaptation to scavenging systems.

The aim of this study was to evaluate mortality through disease and predation rates in relation to egg laying performance. Breeds used were BA, RIR and HB, and the production systems considered were intensive farming and extensive scavenging in rural Malawi.

MATERIALS AND METHODS

A total of 540 pullets were used for this study, 180 from each of the breeds RIR, BA and HB. The purebred chicks were purchased in the market and the HB were bred in the Mikolongwe Government Poultry Farm. The birds were fed commercial (Homestead) poultry starter/grower for the first 4 weeks and a mixture in equal parts of Homestead poultry starter/grower and poultry grower/finisher from the 4th to the 6th week. The 3 breeds of growers were given a vaccine against Newcastle disease at 2 weeks of age and when 6 weeks old were given a booster before being distributed to 3 different locations corresponding to three Agricultural Development Divisions of South Malawi; namely Lilongwe (Town of Lilongwe), Blantyre (Village of Mulange) and Shire Valley (Village of Nsange). Malawi has a sub-tropical climate with a warm rainy season running from November to April, with annual average temperatures in the study areas around 20, 19 and 23°C respectively (Malawi Meteorological Services, 2012).

Each of the locations received 180 pullets, 60 of each of the 3 breeds. These birds were finally divided into two groups of 30 to be used in the field (scavenging conditions) and farm (industrial production). Birds in farming conditions were kept in single experimental cages in buildings with natural ventilation, fed with commercial Homestead poultry grower/finisher ration to week 18 and Homestead poultry layer thereafter; untreated drinking tap water was provided *ad libitum*.

After transfer to the study sites, the chickens in the field were slowly introduced to the new environment, being allowed to roam around from sunrise to sunset under supervision. During this time the growers were supplemented with commercial Homestead poultry grower/finisher ration. *Ad libitum* drinking tap untreated water was also provided in the feeding area.

The following variables were measured: egg weight (g), eggshell strength (kg/cm^2), egg production (total number of eggs in the period), mortality through disease, and predation. Observations were carried out from 24 to 65 weeks and measurements in the eggs taken fortnightly as the last egg closest to the day of observation, in a total of 20 sampled eggs per bird. The mean value for each of the variables in each bird was entered in the analysis. Weights were obtained with a digital balance and eggshell strength with a tensile tester machine (Instron, High Wycombe, Bucks, UK). Predation was distinguished from mortality through disease by evidence of predation and sudden disappearance of healthy birds, while for mortality through disease, the birds were observed to become emaciated and ill in advance of death.

Due to the dichotomous nature of the variables ''mortality through disease'' and ''predation'', several generalised linear models with links belonging to the binomial family were fitted and those with the best fit were chosen. The best fits were achieved with a complementary log log link, as expected, as both mortality through disease and predation are rare events in adult birds. The goodness of fit was evaluated via Deviance and Akaike's Information Criterion (AIC) (SPSS Inc., 2007).

To model the bird mortality, the outcome dichotomy was considered to be died or survived. The predictors used were the categorical variables production system (farm, field) and breed (BA, RIR, HB); and the covariates egg production, egg weight and eggshell strength.

In the predation model, the outcome dichotomous variable was predated or not predated and the predictors used (independent variables) were the factor breed (BA, RIR, HB) and the covariates egg production, egg weight and eggshell strength.

The statistical package used was the SPSS/PASW© Statistics 18 (SPSS Inc., 2007). The Research was approved retrospectively by the Ethics Committee of Hartpury College (Reference: ETHICS2010/22-S).

Table 1. *Parameters of the complementary log log link model fitted to the mortality through disease data. The probability of mortality through disease is modelled in dependency of "breed" and "egg shell strength". Rhode Island Red (RIR) has the higher mortality odds and Hyblack (HB) the lowest, with Black Australorp (BA) in between. "Eggshell strength" has a positive coefficient, and therefore relates positively with the odds of bird mortality*

Variables	β	SE (β)	*P*-value	95% CI (β)	OR ($e^{\beta/2}$)	95% CI ($e^{\beta/2}$)
Intercept	−3·579	1·064	<0·001	[−5·664, −1·494]		
Breed			<0·01			
RIR	0					
BA	−1·224	0·390		[−2·008, −0·481]	0·421	[0·242, 0·712]
HB	−0·932	0·359		[−1·635, −0·229]	0·517	[0·315, 0·851]
Eggshell strength	0·685	0·254	<0·01	[0·188, 1·182]	1·623	[1·143, 2·307]

SE: standard error; CI: confidence interval; OR: odds ratio. The adjusted final model has Deviance 210 and AIC 218. Parameters were transformed by dividing by $\sqrt{2}$, which allows the interpretation of the exponential of these as OR, as in logistic regression models (Rodriguez, 2007). The model is plotted in Figure 1, using the equation: P(mortality through disease) = 1–exp (− exp ($\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$)), where: P(mortality through disease) is the probability of mortality through disease for a given laying hen; β_0 is the intercept; β_1 and β_2 are the regression coefficients calculated for BA and HB; X_1 and X_2 are dummy variables, indicative or not of the breed in the equation; β_3 is the regression coefficient calculated for "eggshell strength"; X_3 is the average shell strength for the eggs of the laying hen.

RESULTS

The mortality through disease model was highly significant (Likelihood ratio Chi-square $P<0{\cdot}01$), had a highly significant ($P<0{\cdot}001$) intercept, a significant ($P<0{\cdot}01$) effect of breed and a significant ($P<0{\cdot}01$) covariate for eggshell strength. No significant effects ($P>0{\cdot}05$) of production system or the covariates egg weight and egg production were detected. The full description of the parameters is presented in Table 1, where the 95% Wald confidence intervals and the odds ratios are also presented.

For the variable "breed", RIR was set as the reference category; BA with the lowest negative coefficient had a decrease in the mortality through disease odds of 57·9%, while the cross breed HB also with a negative coefficient had a decreased mortality through disease odds of 48·3%, both provided there was no change in the eggshell strength.

For eggshell strength, the positive coefficient is explanatory of an increase of 62·3% in the odds ratio of "mortality" per additional unit of shell strength (1 kg/cm^2), indicative of a positive correlation between the probability of mortality through disease and eggshell strength.

The predation model was also significant (Likelihood ratio Chi-square $P<0{\cdot}05$), had a significant ($P<0{\cdot}05$) intercept, with a significant effect of breed ($P<0{\cdot}05$) and a non-significant ($P<0{\cdot}1$) covariate (eggshell strength). Despite the relatively high *P*-value for eggshell strength ($P=0{\cdot}094$), we decided to leave the covariate in the model, as this was highly significant in the mortality through disease model ($P<0{\cdot}01$). The full description of the parameters is presented in Table 2, where the 95% Wald confidence intervals and the odds ratios are also presented.

For eggshell strength, the positive coefficient was explanatory of an increase of 57·2% in the odds ratio of predation per additional unit of eggshell strength (1 kg/cm^2), indicative of a trend for a positive correlation between the probability of predation and eggshell strength.

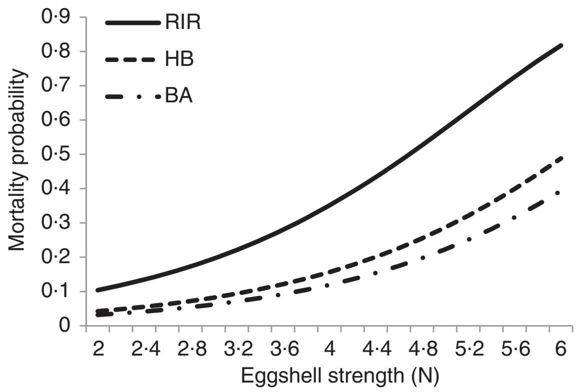

Figure 1. *Mortality through disease probability given by the complementary log log link model fitted. Rhode Island Red (RIR) have higher probability of mortality through disease and Black Australorp (BA) the lowest, with Hyblack (HB) in between. A higher strength in the egg shell increases probability of mortally through disease. This model is valid for eggshell strength values between 2·18 kg/cm^2 and 5·94 kg/cm^2, respectively the minimum and maximum values observed in the data collected and used to fit the model.*

DISCUSSION

Different breeds have different production abilities and behave differently (Jones and Hocking, 1999); with different levels of alertness, flightiness and reaction to predators. These are desirable behaviours in natural environments but not in captivity, and were lost during domestication (Muir, 2003). Tonic immobility is an anti-predatory reaction (Jones, 1986) and selection for low or high levels is possible (Faure and Mills, 1998). Kent and Compton (2011) classify the BA

Table 2. *Parameters of the complementary log log link model fitted to the predation data. The probability of predation is modeled in dependency of "breed" and "egg shell strength". Black Australorp (BA) have the higher odds for being predated, and Hyblack (HB) the lowest, with Rhode Island Red (RIR) in between. "Eggshell strength" has a positive coefficient, and therefore relates positively with the odds for bird being predated.*

Variables in the equation	β	SE (β)	*P*-value	95% CI (β)	OR ($e^{\beta/2}$)	95% CI OR ($e^{\beta/2}$)
Intercept	−4·354	1·662	<0·05	[−7·610, −1·097]		
Breed			<0·05			
RIR	0					
BA	0·914	0·540		[−0·143, 1·972]	1·909	[0·904, 4·033]
HB	−0·613	0·707		[−1·998, 0·772]	0·648	[0·244, 1·726]
Shell strength	0·640	0·382	0·094	[−0·109, 1·390]	1·572	[0·926, 2·672]

SE: standard error; CI: confidence interval; OR: odds ratio. The adjusted final model has Deviance 67 and AIC 86. Parameters were transformed by dividing by $\sqrt{2}$, which allows the interpretation of the exponential of these as OR, as in logistic regression models (Rodriguez, 2007). The model is graphed in Figure 2, using the equation: $P(\text{predation}) = 1 - \exp(-\exp(\beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3))$, where: P(Predation) is the probability of predation for a given laying hen; β_0 is the intercept; β_1 and β_2 are the regression coefficients calculated for BA and HB; X_1 and X_2 are dummy variables, indicative or not of the breed in the equation; β_3 is the regression coefficient calculated for "eggshell strength"; X_3 is the average shell strength value for the eggs of the laying hen.

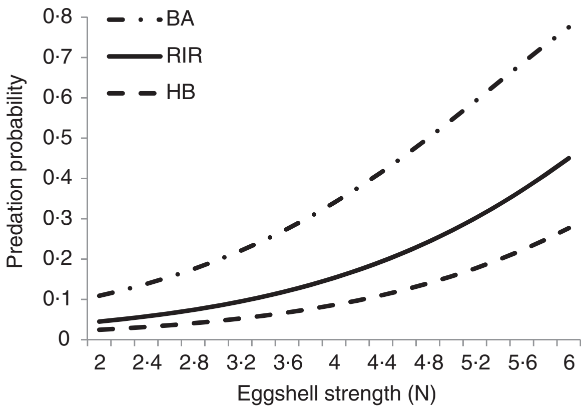

Figure 2. *Predation probability given by the complementary log log link model fitted. Black Australorp (BA) have higher predation probability and Hyblack (HB) the lowest, with Rhode Island Red (RIR) in between and not significantly different from these. A higher strength in the egg shell increases predation probability. This model is valid for eggshell strength values between 2·18kgcm^2 and 5·94kgcm^2, respectively the minimum and maximum values observed in the data collected and used to fit the model.*

breed as broody, calm and docile, and in fact the villagers anecdotally justified higher BA predation with the continuous broody behaviour, using bare soil to lay and spending long periods siting on the eggs. This agrees with Martin (1993) who found that ground-nesting birds suffer greater predation than off ground in shrub and grassland habitats. With regard to mortality through disease, this is lower in BA and HB. It is known that different breeds and strains vary in their resistance to disease (e.g. Permin and Pedersen, 2002) and stress (e.g. Roberts, 2004). In African poultry scavenging systems, mortality is around 85% within the first year of life (Matthewman, 1977; Wilson *et al.*, 1987; Permin and Pedersen, 2002), caused by mismanagement, lack of supplementary feeding, predators and diseases (Pandey, 1992).

Of note in the study is the lack of a significant difference in the mortality through disease between field and farm systems, exposing an unexpected lack of ability to raise biosecurity levels in the industrial systems. In fact industrial systems had natural ventilation without net protection against insects; buildings were not fenced and anybody could approach the buildings including wildlife. The drinking water was from the tap coming from a local dam without treatment and vaccination was done in both field and farm birds.

Modern breeds of layers were selected to produce increased quantities of larger eggs with high feed efficiency and reduced metabolic body mass (Gregory, 2010). If underfed, birds become emaciated (Gregory and Devine, 1999), increasing susceptibility to mortality through disease and predation. With regard to eggshell strength, we found a positive correlation with mortality through disease but no statistical evidence of a change in predation. These results may be explained by the fact that birds with greater eggshell strength are depleted of calcium and become emaciated, with limited mobility and therefore limited foraging or escaping ability, making them susceptible to disease and predation. Hocking *et al.* (2003) found results suggesting that in genetically selected lines of layers, eggshell quality is maintained at the expense of bone strength. The quantity of shell produced is constant and does not increase over time with bigger eggs, and also does not decrease if oviposition is delayed by stress. The negative correlation between temperature and laying due to heat stress is well known, and laying performance in hot weather can only be done at the expenses of bone calcium once intake decreases. Osteoporosis, spinal degeneration or irregularities in intracellular calcium metabolism contributing to inhibited muscular activity and leading to paralysis are possible consequences (Whitehead, 2004).

A limitation of this study is the lack of local breeds to fully access the potential of the exotic breeds used in this trial. There is also some subjectivity in the criteria for distinction between predation and mortality through disease with potential overlap. The results in both situations are similar, but there is a need of further research to fully clarify the relatively weak evidence ($P<0{\cdot}1$) of the eggshell strength in the predation model.

In conclusion, HB crosses deserve attention in the development of breeding programs for scavenging production systems of rural Malawi, due to the lower mortality and, potentially, lower predation, which may be associated with hybrid vigour. A positive relation between high shell strength and mortality through disease and predation was found, and this may be due to depletion of calcium from birds' bones, making them weaker and susceptible to disease and predation.

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RESEARCH

Effect of manual and motorized dental rasping instruments on Thoroughbred's heart rate and behavior

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KEYWORDS:
rasping;
dentistry;
motorized;
manual;
equine;
behavior

Abstract Inadequate research exists to justify the choice of dental tools used in equine prophylactic dentistry; usage is unregulated, with choice usually based on the practitioner's preference. Dental procedures could cause stress (through handling or equipment used), as they are potentially painful if contact is made with soft-tissues. Anecdotally adverse reactions are documented and differ when comparing manual with motorized rasping, particularly during rasping the lower arcade. The study aimed to investigate these claims.

Forty-five Thoroughbreds were divided into groups: (A): manual rasping, (B): motorized rasping, and (C): control. Horses were accustomed to prophylactic dentistry (6-12 months previously) and required a routine float. A standardized protocol was used; heart rate (HR) and scale-graded interactive behavioral responses (BR) were recorded to evaluate subjects' stress and pain pre-, peri-, and posttreatment.

Increased HR and BR were observed in group A compared with groups B and C ($P < 0.001$). Motorized rasping produced a significant increase in HR ($P < 0.001$) and BR ($P < 0.001$) from the controls. The results support postulation of higher sensitivity in the lower arcades, with these arcades exhibiting significantly higher HR and BR in both motorized and manual groups ($P < 0.001$). "Bit-seating" recorded higher responses (HR and BR: $P < 0.001$) compared with routine rasping.

The study could inform routine dentistry; motorized rasping produced less-reactive horses, potentially providing a safer working environment for dental technicians and increased welfare for their equine patients.

Introduction

Equus caballus has originated from *Hyracotherium*, which fed on soft leafy vegetation and possessed brachydont teeth suitable for this form of forage (Baker and Easley, 2005). Climatic events changed the topography of the primitive horses' environment and adaptation to the coarse forage present facilitated morphological development of dentition, resulting in hypsodont cheek teeth that continually erupt to provide an effective grinding surface (Dixon, 1993; Dacre, 2006; Du Toit, 2006). Domestication of the horse has advanced variation in dental profiles of equine breeds (Johnson and Porter, 2006). Modern enforced dietary regimes (often considerably less than the 100% forage of a feral equid and of a softer consistency requiring reduced mastication) (Stubbs, 2004; Knottenbelt, 2005) negate the requirement for continual eruption (Dixon and Dacre, 2005). Equally, the reduced occlusal wear of the cheek teeth often produces abnormal wear patterns negatively

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impacting functionality (Dixon, 1993; Stubbs, 2004). Therefore, prophylactic dentistry is an essential aspect of routine care of the modern performance horse, and additional performance dentistry protocols such as rostral profiling or bit seating are common place in the equine athlete.

A recent review by Dixon et al. (2005) found that 83% of horse owners organized regular dental treatment for their horses. Normally, an equine dental examination will comprise removal of buccal focal overgrowths (rasping) to restore fully functional occlusal contact or occlusal equilibrium. Rasping can be conducted either manually or by using motorized tools, the latter is advocated for removal of large buccal overgrowths (Dixon and Dacre, 2005), and sedation of the patient is often recommended (Burnett, 2005). Rostral profiling or "bit-seating" involves rounding of the rostral aspect of the second premolars (Kempson et al., 2003; Dacre, 2006), and it is a complimentary procedure often used in performance horses to prevent buccal tissue entrapment with the bit and/or pain from the fitting of the bit (Johnson, 2007).

Welfare of the horse is paramount throughout dental procedures not only as an ethical concern but also to facilitate a safe working environment and prevent injury to horse and dental technician/veterinary surgeon. Knowledge of tooth morphology and dental anatomy is essential for practitioners to prevent excessive removal of enamel and subsequent exposure of the vascular and neural pulp, as well as sensitive dentine, which would cause pain and long-term damage to the horse (Kempson et al., 2003; van den Enden, 2008; Huthmann et al., 2009). Veterinary procedures have the potential to compromise equine welfare due to their aversive nature (Veisser and Boissy, 2007), and anecdotally negative reactions to routine dental treatments have been documented, suggesting that the use of manual tools is more likely to compromise welfare than using motorized tools. Increased sensitivity within patients is also reported by practitioners during rasping of the lower teeth, affecting their ability to safely and effectively perform treatment (Carmalt, 2007; van Forest, 2008).

Because of advancements in the equine dental industry, both manual and motorized hand floats (rasping tools) for the removal of overgrowths of the clinical crown have become available (Easley, 1998; Baker and Allen, 2002). Manufacturers of modern motorized equipment claim that they are more efficient, have increased specificity in action, and are more ergonomic for the operator in comparison with hand floats (Scoggins, 2004; Klugh, 2005). However, no scientific validation has been undertaken for use in either variety of tool with respect to stress and pain experienced by the horse. Therefore, choice of tools is primarily based on practitioner's personal preference rather than established practical protocols or welfare guidelines (Baker and Allen, 2002; van Forest, 2008). We aimed to investigate whether performance horses experienced differing levels of stress or pain during routine dental floating using manual and motorized tools.

Materials and methods

The sample population comprised Thoroughbred National Hunt racehorses ($n = 45$; 74% geldings and 26% mares) aged 7.44 ± 1.01 years (range: 6-9 years). To avoid bias, all participants were required to: have been housed on the same yard under consistent management conditions (diet, exercise, and prophylactic dental care) for a minimum of 1 year before treatment, have been exposed to manual and motorized dental equipment before treatment (within 6-12 months), not have previously required sedation for dental treatment, and clinically required routine dental floating during the period of the study. Horses were allocated into 1 of 3 trial groups, using random systematic sampling. Group A horses ($n = 15$) were treated with manual equipment, hand floats with solid tungsten carbide blades. Group B horses ($n = 15$) were treated with the motorized Dearson Power Tool (Dearson Tools Ltd., Birmingham, United Kingdom). Group C horses ($n = 15$), the control group, received no dental treatment.

Ethical consent for the research was provided by the University of the West of England's (Hartpury College) Ethics Committee. Horses that reacted adversely to treatment or were influenced by external factors, including noise, treatment environment, and movement in the surroundings; other horses or people; or change of equipment or procedure were proposed to be eliminated from the study. However, no horses were required to be withdrawn from the study for these reasons.

Many studies (e.g., Visser et al., 2002; Rietmann et al., 2004a) have used heart rate (HR) as a reliable physiological measure of stress within the horse. HRs for each horse were recorded using a Polar T51H (Polar Electro, Warwick, England, United Kingdom) uncoded transmitter and wrist unit, Polar 5810i, in conjunction with a wireless receiver using methodology previously validated by Clegg et al. (2008) and Waran and Cuddeford (1995). It has been established that the behavior of a horse changes in response to pain (Valverde and Gunkel, 2005; Bussieres et al., 2008). Behavioral reactions (BRs) to the environment were recorded to identify individual's pain tolerance to dental procedures and equipment type, using observations of body posture (eye, head, and ear position), locomotory activity, and response to the equine dental technician (EDT) and dental stimuli (tools). BRs were graded using an adapted linear scale (Table 1).

A constant assessor, previously trained in the protocols used, recorded HR and BRs. Horses were allowed to familiarize themselves with the assessor within the confined stable environment for 5 minutes before the entry of the EDT. A 10-minute habituation period was then allowed (Rivera et al., 2002; Waiblinger et al., 2004), followed by initial examination by the EDT, and then attachment of the HR monitor to the surcingle and the horse. The unfamiliar event could have caused an individual reaction in terms of excitement, fear, or stress (physiologically or

Table 1 Composite numerical pain scale for visual scoring of behavioral activity in horses with pain (Pritchett et al., 2003; Driessen and Zarucco, 2007)

Behavior category	1	2	3	4
Gross/obvious pain behaviors	No pain	Average pain	Moderate pain	Severe pain
Head position	Relaxed	Raised, occasional movement	Variable/moving away from discomfort	Still/tense, either severely elevated or dropped
Ear position	Forward, free moving	Forward/still	Backward/tensed	Flat backward
Eye	Relaxed	Alert	Wider/Pupils still	Widened/displaying whitened corners of the eye
Positioning in the stable	Toward the front (door)	Centered in the stable, still looking toward the door	Moving to the sides, away from the door	Back/corner of the stable
Spontaneous locomotion	Free/voluntarily movement of feet/body	Occasional movement	Reluctant to move/lift feet	None/defensive/muscle tremors
Response to EDT and stimuli of dental tools	Approaches EDT/stimuli, ears forward, investigating	Looking forward, ears forward	Backing off/warning of aggression with ears moving back, feet lifting	Defensive/aggressive positioning, ears flat backward, strike with feet

EDT, equine dental technician.

behaviorally). Habituation allowed the horse to acclimatize and stabilize its behavior and HRs (to resting level) before the collection of data (Voss et al., 2002; Young, 2003; Rietmann et al., 2004a) HR and BR data were recorded every 15 seconds for each quadrant or bit seat, in accordance with timing protocols devised by Waran and Cuddeford (1995). Posttreatment, dental equipment was removed and the EDT left the stable.

A single British Equine Veterinary Association (BEVA)-qualified EDT participated and administered dental treatments. The BEVA qualification, approved by Department for Environment, Food, and Rural Affairs and the Royal College of Veterinary Surgeons, certifies ethical unsedated treatment (RCVS, 2005; BEVA, 2010). Treatments occurred over a 2-month period. Horses were housed in their normal stable for the duration of the treatment and caught and handled by the assessor and EDT only, as the presence of a recognizable human has been shown to cause fluctuations in behavior and HR (Visser et al., 2002; Lansade et al., 2008).

Following habituation, each subject was fitted with a Haussmann speculum, and then underwent a 10-minute pretreatment dental examination conducted by the EDT to chart abnormalities (using the Triadan system) (Floyd, 1991) and establish treatment. A standardized routine protocol had been approved by the EDT (Figure 1); each stage was standardized, 5 minutes of rasping were allocated for each quadrant, with the entire procedure taking 40 minutes.

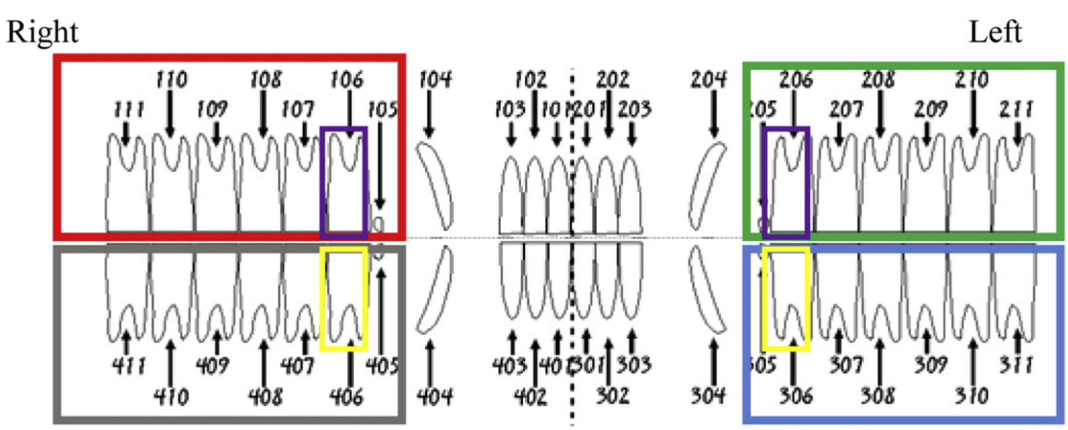

Figure 1 Rasping order of the dental examination, using a modified Triadan system of dental nomenclature: First, lower left quadrant (blue); second, lower right quadrant (grey); third, bit seating lower left second premolar (306) then lower right second premolar (406) (yellow); fourth, upper left quadrant (green); fifth, upper right quadrant (red); and sixth, bit seating upper left second premolar (206) then upper right second premolar (106) (purple) (Floyd, 1991). For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

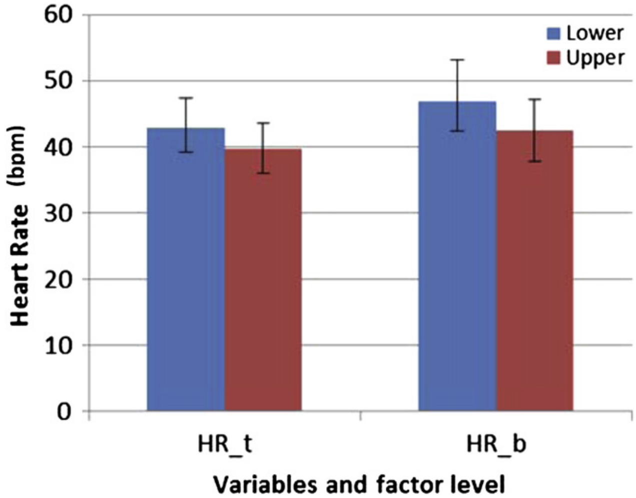

Figure 2 Heart rate means and standard deviations when rasping teeth (HR_t) or bit seating (HR_b) in the upper and lower quadrants.

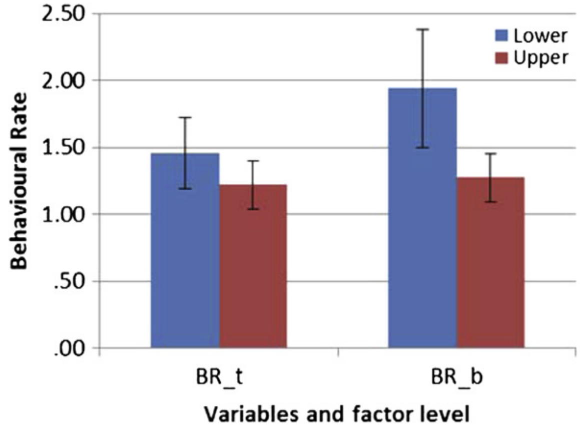

Figure 3 Behavioral response rate means and standard deviations when rasping teeth (BR_t) or bit seating (BR_b) in the upper and lower quadrants.

Horses in group C (control) underwent the same standard pretreatment dental examination and habituation period as groups A and B. HR and BR data were recorded for a comparative 10-minute period during which the horse wore the speculum and the EDT mimicked dental treatment of the quadrants by opening/closing the mouth every 5 minutes.

HR and BR data were collected every 15 seconds during the 5 minutes allocated to each quadrant and bit seat, for a total of 20 measurements for each in every quadrant and bit seat. Means were then calculated for these for each animal (4 quadrants and 4 bit seats, independently), which were analyzed using PASW 18.0 (IBM, USA). The data had complete groups, were balanced, and only fixed factors were considered. Analysis of variance (ANOVA) tests were used to analyze the 4 variables considered: heart rate for routine rasping (HR_t), heart rate for bit seat work (HR_b), behavioral rate for routine (BR_t) work, and behavioral rate for bit seat work (BR_b). The factors and respective levels considered were as follows: "position" (upper, lower) referring to quadrants, "side" (left, right) referring to left and right quadrants, respectively, and "process" (manual, motorized, and control) referring to the rasping tool used. The variables HR_t and HR_b had a distribution that approached normal (Kolmogorov–Smirnov: $P = 0.027$ and $P = 0.083$, respectively), whereas BR_t and BR_b did not (Kolmogorov–Smirnov: $P < 0.001$ and $P < 0.01$, respectively). The homogeneity of variances was not met for any of the variables (Levene's $P < 0.001$); therefore, different strategies were used to divert from the violation of assumptions.

Repeated measures ANOVA was used with each of the variables for the factors "position" and "side." The Greenhouse–Geisser reading was given, after sphericity could not be assumed. The factor "side" was abandoned due to lack of significance ($P > 0.05$). 1-way ANOVA was used with each of the variables for the factor "process." Tamhane's T2 was used as post hoc test, after the variables did not show homogeneity of variances. Repeated measures ANOVA was used to analyze and compare "routine rasping" with "bitseat" with regard to HR and BRs. Again the Greenhouse–Geisser reading was given, as sphericity could not again be assumed. The residuals were checked after the ANOVAs, and they did not show any deviations from normality that could not be accounted for by the robustness of ANOVA.

Results

The factor "position" was analyzed with regard to the 4 different variables considered, and significant differences were found between the upper and lower quadrants ($P < 0.001$), with the HR and BR rate both being elevated when the lower quadrants were being rasped. The differences can be observed in Figures 2 and 3.

The factor "process" was analyzed with regard to the 4 different variables considered, and the results are summarized in Table 2. Significant differences were found between groups, with the "manual" horses constantly displaying higher mean HRs ($P < 0.001$) and BR rates ($P < 0.001$) in comparison with the other groups. The "motorized" group recorded consistently reduced HRs and BR rates ($P < 0.001$), when rasping the teeth, from group A, but were increased from data recorded in the control group.

The impact of routine rasping of teeth in comparison with bit seating was also investigated. Significant differences were found for both BR rate ($P < 0.001$) and HR ($P < 0.001$), with horses exhibiting increased HR (mean: 44.73 ± 11.11 bpm) and BR scores (mean: 1.61 ± 0.75) during bit seating in comparison with routine rasping (HR mean: 41.31 ± 8.42 bpm and BR mean: 1.34 ± 0.47).

Discussion

The modern performance horse, epitomized by the Thoroughbred racehorse, is stabled and fed a high concentrate,

Table 2 Mean and standard deviations of heart rate and behavior responses during routine and bit seat rasping in the 3 examined groups: horses treated with manual equipment (n = 15), with the motorized Dearson Power Tool (n = 15), or received no dental treatment (controls; n = 15)

Variable	Factor	Levels	Means	Standard deviation	*P* value
Heart rate—teeth (bpm)	Process	Control	33.47[a]	2.18	<0.001
		Manual	52.01[b]	4.08	
		Motorized	38.45[c]	2.53	
Heart rate—bit seat (bpm)	Process	Control	33.20[a]	1.89	<0.001
		Manual	58.60[b]	4.91	
		Motorized	42.40[c]	3.30	
Behavior responses—teeth	Process	Control	1.08[a]	0.10	<0.001
		Manual	1.82[b]	0.52	
		Motorized	1.12[a]	0.18	
Behavior responses—bit seat	Process	Control	1.06[a]	0.08	<0.001
		Manual	2.33[b]	0.75	
		Motorized	1.43[c]	0.51	

Note: Comparisons among groups were made by using Tamhane's T2 post hoc tests.
bpm, beats per minute.
[a,b,c]Means with different letters in superscript differ significantly at the level of $P < 0.05$.

low forage diet that does not provide sufficient wear to counteract the continual eruption of hypsodont cheek teeth. Prophylactic dentistry is necessary to remove the resultant focal overgrowths, with powered rotary abrasive tools becoming more popular to facilitate this process. This field study examined the use of both manual and motorized tools during routine rasping to discover whether either or both presented a welfare conundrum to the horse, and whether topography (upper or lower arcade) influenced perceived sensitivity to floating.

Variance in the mean HR of the trial groups was recorded, with analysis confirming a high significance between mean HR and group for routine rasping. Behavioral observations demonstrated the same pattern, and group allocation was significantly associated with increased responses (manual> motorized> control). The results also suggest that the process of manual rasping is a more potent stressor to the horse than motorized rasping, although both represent significant insults compared with the control group. All groups exhibited an increased HR from resting levels (28-36 bpm: according to Voss et al., 2002; Young, 2003; Rietmann et al., 2004a), suggesting that the action of examination alone or combined with treatment does produce stress or flight responses in the horse, similar to the results of the current study. Previous studies (Porges, 1995; Bachmann et al., 2003) have suggested that horses perceive dental treatment as a stressful event and physiological outputs include adaptations to the sympathetic nervous system (tachycardia, tachypnea, sweating) in preparation for a fight or flight response. It has been postulated that horses within a confined environment (as here, restrained in a stable) may externalize physiological outputs due to the presence of a stressor as overt behaviors (Mal et al., 1991; Chapman et al., 2008). However, evaluation of behavior is a subjective assessment tool and in this study BRs, although present, are less pronounced than physiological data. This could be due to individual differences in pain perception and learnt BRs (McGreevy et al., 1995; von Borell et al., 2007), misinterpretation of behavior by the assessor, or the subjective nature of using a scale to grade behavior. The use of more than 1 assessor of BRs in this study could have reduced bias through assessment of interassessor reliability. Future work in this area would also benefit from the assessment of HR variability (the monitor used here did not facilitate this) during dentistry, as this has been proposed to produce more accurate assessment of this parameter (Rietmann et al., 2004b).

Chapman et al. (2008) suggested that horses remember past experiences and utilize them to respond to novel experiences. All individuals in this study had previously experienced dental treatments, and it could be assumed, therefore, that a degree of anticipation influenced physiological and behavioral outputs (Veisser and Boissy, 2007). The lack of sedation in previous dental treatments for participants may have influenced both the HRs and BRs recorded, as sedation is routinely advocated during the use of motorized equipment (Burnett, 2005; Dixon and Dacre, 2005) to compensate for the noise and novel experience it presents. The horses in this study had previous experience of motorized tools and were deemed by the experienced EDT to not require sedation, as this procedure was a routine prophylactic rasp and none exhibited any excessive tooth growth that would necessitate extended (>30 minutes) treatment, which would in turn require sedation. However, the absence of sedation during previous experiences may have resulted in increased stress (not perceived by the EDT), which could have influenced individual learnt BRs and HR displayed in the current study. Disorders of the mouth can discretely cause pain due to compromised mastication or mucosal ulceration (Allen, 2004; Tell et al., 2008), potentially eliciting a response to dentistry (Johnson and Porter, 2006). The presence of established coping mechanisms cannot be discounted but could provide an alternative rationale for the reduced BRs observed in comparison with HR, as research has shown that horses may not exhibit BRs to either a stressful or painful stimulus while retaining an innate physiological reaction (Visser et al., 2002; Rietmann et al., 2004a). The increased parameters during treatment from resting values in the controls could be due to the presence of the speculum and supports the idea that horses experience an anticipatory response after the speculum is fitted. Alternatively, the presence of the speculum could be uncomfortable and could elicit a reduced pain response in subjects already coping with underlying dental pathology, as all subjects' dentition did require clinical attention.

Anecdotal reports of increased equine anxiety during rasping of the lower dental arcades in comparison with the upper arcades have been attributed to the lack of support to the mandible and administration of excessive downward force, thereby applying strain to the temporomandibular joint (Dixon, 1993; Baker, 2002; Dixon and Dacre, 2005) and causing distress. Our results lend weight to this hypothesis, as horses in this study exhibited significantly higher mean HRs and increased BRs during routine rasping of the lower arcades. It should be noted that dentists tend to rasp the lower quadrants first due to the anecdotal musings mentioned previously. It could be postulated that in addition to extension of the temporomandibular joint, horses are required to counterbalance the force applied during lower arcade rasping through engagement of the muscles of the head and neck, which may also contribute to these results. Further research is warranted to fully validate the findings and to investigate further why this is the case.

The highest values for mean HR and increased BRs were recorded during bit seating and followed the same profile as routine rasping, with a significant association found between HR and BR to group allocation (manual > motorized). Bit seating is a common component of modern prophylactic dentistry; however, no standardized protocol exists. According to Dixon (2002) and Kilic et al. (1997), the anatomy of the second premolar predisposes it to pulpar insult and hyperthermia of the pulp (Dixon, 2002; Carmalt, 2007) during rasping (particularly with motorized tools). Pulp is a neural tissue and pulpar exposure or thermal insult could enhance the propensity for pulpitis (Johnson and Porter, 2006; Johnson, 2007) and therefore subsequent pain or stress in an individual. Our results suggest the process is an enhanced stressor in comparison with routine rasping, but care over interpretation should be exhibited due to sample size and breed as well as the inability to measure the influence of previous dental work.

Manual and motorized tools differ in their mode of action, noise levels generated, and vertical forces applied to the tooth. Manual rasping has a push–pull action requiring considerable downward exertion on the tooth, resulting in comparatively high noise levels and the risk of damage to soft-tissue from the blade (Litt, 1996; Price et al., 2002; Allen, 2003; Kempson et al., 2003). Motorized tools (represented in this study by the Dearson Power Tool) are considered to be quieter, they have a safety shutdown mechanism to prevent soft-tissue damage, and the mechanical action requires less force to facilitate removal of the occlusal surface of the tooth. Manual blades, in contrast to the motorized burr, are thought to remove excessive amounts of tooth, exposing dentine and/or pulp to the oral environment (Kempson et al., 2003; van den Enden, 2008; Huthmann et al., 2009). The heightened sensitivity of dehydrated dentine has been suggested to contribute to pain experienced during treatment (Johnson and Porter, 2006). It is proposed that the differing qualities of the tools contributed to the variance seen in the parameters under investigation; the reduced response to the motorized tool would suggest that this is perceived as a reduced stressor than its manual equivalent, even in the unsedated patient.

Individual variance in mean HR and BR data occurred within all groups; therefore, caution should be shown in an umbrella approach to analysis of the results. It is recognized that individuals have a specific pain tolerance and pain threshold (Bussieres et al., 2008) that should be considered by the dental practitioner during interpretation and application to practice. Thoroughbreds have also been found to exhibit exaggerated behavioral and physiological responses when subjected to an intensive management regime (Visser et al., 2002; Lansade et al., 2004) and are considered to be more highly reactive to stressors than other breeds of horse (Lloyd et al., 2008; Waran et al., 2008). Therefore, it would be of benefit to repeat the study with a wider variety of breeds to ensure conclusions formed are transferable.

Conclusion

To our knowledge, this study was the first to examine whether horses experience variable stress levels, via physiological and behavioral indices, during prophylactic dentistry using manual and motorized rasping tools. It appears that Thoroughbreds exhibit a stress response to prophylactic dentistry, but this is reduced by the use of motorized rasping tools in comparison with manual floats. Further investigation into the differential response between lower and upper dental arcades and during bit seating is required to evaluate whether trends observed are of relevance to the dental practitioner. The use of motorized rasping tools for routine dentistry could produce a less reactive horse, thereby improving equine welfare and providing a safer working environment for the practitioner.

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Appendix II: Statements and Declarations

Declaration relating to research degrees at another institution (accordingly to Anglia Ruskin University Research Degrees Regulations 2015, Part B, paragraph 4.1, item e; subject to Part A, paragraph 11.7)

I declare that this work submitted in whole or in part has not been accepted for a research degree at any other university.

NAME: Fernando Jorge Ribeiro da Mata

SIGNATURE:

Fernando Jorge Ribeiro da Mata

DATE: 01/08/2016

Declaration relating to ethics (accordingly to Anglia Ruskin University Research Degrees Regulations, Part B, paragraph 4.1, item f)

I declare that the published papers part of this thesis received ethical approval, where required, at the time the research was undertaken.

NAME: Fernando Jorge Ribeiro da Mata

SIGNATURE: Fernando Jorge Ribeiro da Mata

DATE: 01/08/2016

Statement identifying where, when and over what period the research contributing to the published works was undertaken (accordingly to Anglia Ruskin University Research Degrees Regulations 2015, Part B, paragraph 4.1, item c)

The following papers part of this thesis were written and resulted from research done at Hartpury College, an associated Faculty of the University of the West of England between January 2011 and August 2013:

1. Mata, F., Mwakifuna B., (2012) Comparative mortality and predation in relation to egg production traits of Rhode Island Red, Black Australorp and Hyblack laying hens in scavenging production systems of rural Malawi. *British Poultry Science*. 53 (5), pp.570-575.
2. Mata, F., Williams J., Marks F. (2012) Investigation of factors associated with the probability of racehorses being pulled up in steeplechase races at Cheltenham racetrack. *Comparative Exercise Physiology*. 8 (2), pp.95-101.
3. Williams J., Parrot R., Da Mata, F., (2012). Effect of manual and motorised dental rasping instruments on Thoroughbred’s heart rate and behavior. *Journal of Veterinary Behavior: Clinical applications and research*. 7 (3), pp.149-156.

The following papers part of this thesis were written and resulted from research done at the School of Agriculture, Food and Rural Development of the Newcastle University, between September 2013 and August 2015:

1. Mata, F. (2015) The choice of diet affects the oral health of the domestic cat. *Animals*. 5 (1), 101-109.
2. Mata, F., Johnson, C., Bishop, C. (2015) A cross-sectional epidemiological study of prevalence and severity of bit-induced oral trauma in polo and race horses. *Journal of Applied Animal Welfare Science*. 18 (3), pp.259-268.
3. Mata, F. (2014a) Evaluation of horse fitness for exercise: the use of a logit-log function to model horse post-exercise heart rate recovery. *Journal of Equine Veterinary Science*. 34 (9), pp.1055-1058.

4. Mata, F. (2014b) Analysis of predisposition factors for limb amputation in dogs with survival analysis in those diagnosed with appendicular cancer. *The Veterinary Nurse*. 5 (7), pp.406-411.
5. Mata, F. (2013) Mastitis vaccination in dairy cattle: a meta-analysis of field case-control trials. *Revista Portuguesa de Ciências Veterinárias*. 108 (585-586), pp.17-22.
6. Mata, F., Lam, A. (2013) Investigating the relationship between feed and helminthic burden of captive birds of prey in Hong Kong. *Zoo Biology*. 32 (6), pp.652-654.

NAME: Fernando Jorge Ribeiro da Mata

SIGNATURE: Fernando Jorge Ribeiro da Mata

DATE: 01/08/2016

Statement indicating the extent of the contribution by collaborating authors with reference to the contribution to design, analysis, conduct of the research and writing up of the publication (accordingly to Anglia Ruskin University Research Degrees Regulations 2015, Part B, paragraph 4.1, item d)

There are four papers where I am the sole author and therefore I am claiming 100% authorship:

7. Mata, F. (2015) The choice of diet affects the oral health of the domestic cat. *Animals*. 5 (1), pp.101-109.
8. Mata, F. (2014a) Evaluation of horse fitness for exercise: the use of a logit-log function to model horse post-exercise heart rate recovery. *Journal of Equine Veterinary Science*. 34 (9), pp.1055-1058.
9. Mata, F. (2014b) Analysis of predisposition factors for limb amputation in dogs with survival analysis in those diagnosed with appendicular cancer. *The Veterinary Nurse*. 5 (7), pp.406-411.
10. Mata, F. (2013) Mastitis vaccination in dairy cattle: a meta-analysis of case-control trials. *Revista Portuguesa de Ciências Veterinárias*. 108 (585-586), pp.17-22.

There are five papers written in collaboration:

4. Mata, F., Mwakifuna B., (2012) Comparative mortality and predation in relation to egg production traits of Rhode Island Red, Black Australorp and Hyblack laying hens in scavenging production systems of rural Malawi. *British Poultry Science*. 53 (5), pp.570-575.

The study design was done in collaboration by the two authors. My collaborator Bernard Mwakifuna collected the data and I have done all the rest: introduction, data analysis, results and discussion. I am therefore claiming 80% of the paper.

5. Mata, F., Williams J., Marks F. (2012) Investigation of factors associated with the probability of racehorses being pulled up in steeplechase races at Cheltenham racetrack. *Comparative Exercise Physiology*. 8 (2), pp.95-101.

The data was initially collected for a different study which also lead to a publication. I have however used the same data in a different study. Different dependent variable and therefore different research questions. We were all involved in the design. Ffion Marks has collected the data, Jane Williams has co-written the discussion with me and I have done the rest: introduction, data analysis and results section. I am claiming 50% of the paper.

6. Williams J., Parrot R., Da Mata, F., (2012). The effect of manual and motorised dental rasping instruments on Thoroughbred heart rate and behavior. *Journal of Veterinary Behavior: Clinical applications and research*. 7 (3), pp.149-156.

I have done the data analysis and the results section of the paper with a small input in the discussion. I am therefore claiming 20% of the paper only.

7. Mata, F., Lam, A. (2013) Investigating the relationship between feed and helminthic burden of captive birds of prey in Hong Kong. *Zoo Biology*. 32 (6), pp.652-654.

The data was initially collected for a different study. I have however used the same data in a different study. Different dependent variable and therefore different research questions. Alice Lam has collaborated in the study design and collected the data. I have done the rest: introduction, data analysis, results and discussion. I am claiming 80% of the paper.

Mata, F., Johnson, C., Bishop, C. (2015) A cross-sectional epidemiological study of prevalence and severity of bit-induced oral trauma in polo and race horses. *Journal of Applied Animal Welfare Science*. 18 (3), pp.259-268.

The data was collected by Charlotte Bishop and Claire Johnson. Claire has also revised the paper with a small contribution in the discussion. We have all designed the study and I have done the introduction, methods, analysis, results and discussion sections. I am claiming 70% of the paper.

NAME: Fernando Jorge Ribeiro da Mata

SIGNATURE: Fernando Jorge Ribeiro da Mata

DATE: 01/08/2016

Appendix III: Chronology of all publications by Fernando da Mata

Note 1: My name in the publication appears in bold; full references in bold are the publications part of this thesis

Note 2: I use my first and last name in my publications (Mata, F.) dropping the "da" and the middle names. However in some publications submitted by co-authors the "da" may appear in several forms such as in "DaMata, F.", "da Mata, F." and "Da Mata, F.". My middle name initials may also be present such as in "da Mata, F. R.".

Mata, P., Gibson, K., **Mata, F.** (2016) An exploration of the therapeutic effect of working in the woodlands: A case study of young adults in a Special Educational Needs College. *Journal of Research in Special Education Needs*.

Raine, A., Douglas, C., **da Mata, F.** (2016) Exploring the effectiveness of hydrotherapy to rehabilitate gait in dogs with joint problems. Proceedings of the British Society of Animal Science Annual Conference. 6-7 April, Chester, UK. In: *Advances in Animal Biosciences, Animal Science for a Sustainable Future*. **7** (1), p.31.

Douglas, C., **Mata, F.**, Menem, G. (2015) Hip scoring for canine hip dysplasia: A comparison of British and German breeding strategies. Proceedings of the British Society of Animal Science Annual Conference. 14-15 April, Chester, UK. In: *Advances in Animal Biosciences, Science into Practice: science with impact.* 6 (2), p.227.

Houghton, R., Douglas, C., **da Mata, F.** (2015) A comparison of mastitis incidence in conventionally managed and organic dairy cows on the same farm. Proceedings of the British Society of Animal Science Annual Conference. 14-15 April, Chester, UK. In: *Advances in Animal Biosciences, Science into Practice: science with impact.* 6 (2), p.184.

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Mata, F. (2014) Analysis of predisposition factors for limb amputation in dogs with survival analysis in those diagnosed with appendicular cancer. *The Veterinary Nurse.* 5(7), pp.406-411.

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Onyango, J., **Mata, F.,** McCormick, W., Chapman, S. (2014). Epidemiology of orf in sheep flocks across England. *Proceedings of the Society for Veterinary Epidemiology and Preventive Medicine Conference (SVEPM)*: Annual Meeting. 26-28 March, Dublin, Ireland.

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Williams, J., Smith, K., **DaMata, F.** (2014) Risk factors associated with horse-falls in UK Class 1 Steeplechases: 1999-2011. *International Journal of Performance Analysis in Sport*. 14 (1), pp.148-152.

Mata, F. (2013) Mastitis vaccination in dairy cattle: a meta-analysis of field case-control trials. *Revista Portuguesa de Ciências Veterinárias*. 108 (585-586), pp.17-22.

Mata, F., Lam, A. (2013) Investigating the relationship between feed and helminthic burden of captive birds of prey in Hong Kong. *Zoo Biology.* 32 (6), pp.652-654.

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Mata, F., Williams J., Marks F. (2012) Investigation of factors associated with the probability of racehorses being pulled up in steeplechase races at Cheltenham racetrack. C*omparative Exercise Physiology*. 8 (2), pp.95-101.

Twigg-Flesner, A., **Mata, F.**, Greening, L., Bourbon, J. (2012). The effects of two endocrine treatments on the size of ovulatory follicles in Lusitano mares in Portugal. Proceedings of the British Society of Animal Science Annual Conference. 24-25 April Nottingham, UK. In: *Advances in Animal Biosciences, healthy food from healthy animals*. 3 (1), p.121.

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